

**MORPHOLOGICAL AND MOLECULAR EVALUATION OF
THERMOSENSITIVE GENETIC MALE STERILE (TGMS)
LINES AND THEIR HETEROTIC COMBINATIONS IN
RICE (*Oryza sativa* L.)**

Thesis

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By

PARDEEP KUMAR

M.Sc. (Ag.) Genetics and Plant Breeding

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*Pantnagar
August, 2015*


(Pardeep Kumar)
Author

CERTIFICATE

This is to certify that the thesis entitled “**MORPHOLOGICAL AND MOLECULAR EVALUATION OF THERMOSENSITIVE GENETIC MALE STERILE (TGMS) LINES AND THEIR HETEROTIC COMBINATIONS IN RICE (*Oryza sativa* L.)**” submitted in partial fulfillment of the requirements for the degree of **Doctor of Philosophy** with major in **Genetics and Plant Breeding** and minor in **Molecular Biology and Biotechnology**, of the college of Post-Graduate Studies, G.B. Pant University of Agriculture and Technology, Pantnagar, is a record of *bona fide* research carried out by **Mr. Pardeep Kumar, Id. No. 44160**, under my supervision and no part of the thesis has been submitted for any degree or diploma.

The assistance and help received during the course of this investigation have been duly acknowledged.

Pantnagar
August, 2015



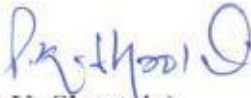
(M. K. Nautiyal)
Chairman
Advisory Committee

CERTIFICATE

We, the undersigned, members of the Advisory Committee of **Mr. Pardeep Kumar, I.D. No. 44160**, a candidate for the degree of **Doctor of Philosophy** with major in **Genetics and Plant Breeding**, and minor in **Molecular Biology and Biotechnology** agree that the thesis entitled “**MORPHOLOGICAL AND MOLECULAR EVALUATION OF THERMOSENSITIVE GENETIC MALE STERILE (TGMS) LINES AND THEIR HETEROTIC COMBINATIONS IN RICE (*Oryza sativa* L.)**” may be submitted in partial fulfilment of the requirements for the degree.


(M. K. Nautiyal)
Chairman
Advisory Committee


(Surendra Singh)
Member


(P.K. Shrotria)
Member


(N. K. Singh)
Member


(A.K. Gaur)
Member


Head of Department
Ex-officio member

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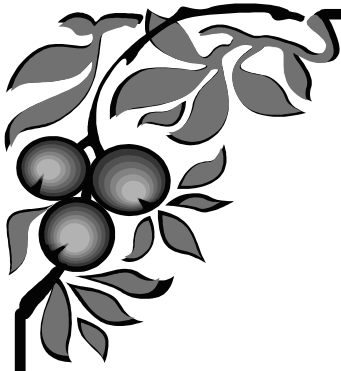
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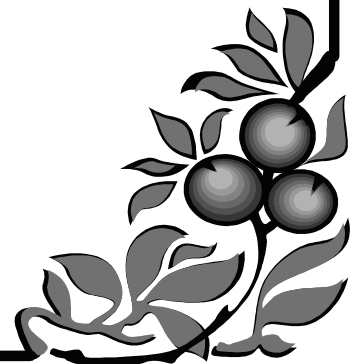
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ABBREVIATIONS

EDTA	Ethylene di amine tetra acetic acid
ddW	Double distilled water
RT	Room temperature
μ M	Micromole
mM	Milimole
O.D.	Optical density
μ g	Micro gram
ng	Nano gram
DNA	Deoxy ribonucleic acid
dNTPs	Deoxy-ribonucleoside tri phosphate
PCR	Polymorphic chain reaction
SSR	Simple Sequence Repeat
TAE	Tris-acetate EDTA
TE buffer	Tris EDTA buffer
Bp	Base pair
CTAB	Cetyl trimethyl ammonium bromide
TGMS	Thermosensitive genetic male sterility
CGMS	Cytoplasmic Genetic Male Sterility
PGMS	Photosensitive Genetic Male Sterility
PTGMS	Photo-thermosensitive Male Sterility
CMS	Cytoplasmic Male Sterility



Introduction



Rice (*Oryza sativa* L.) is a plant belonging to the family of grasses, gramineae (Poaceae). It is one of the three major food crops of the world and forms the staple diet of about half of the world's population. India has a long history of rice cultivation. Rice is the major source of calories for more than half of the global population. It contains 6-12% protein, 70-80% carbohydrate, 1.2-2.0% mineral matter and significant content of fats and vitamins. Rice supplies 23% of global human/ capita energy and 16% of per capita protein requirements. The geographical distribution of rice extends from 39° N latitude (Australia) to 45° N latitude (Japan) and 50° N latitude (China). In India, it stretches from 8° N latitude to 34° N latitude. Globally, it stands first in area and second in production, after China. It contributes 21.5 percent of global rice production. India produces 106 million tons of rice (**Anonymous, 2013**). Rice is grown in all the states in the country. It is cultivated over an area of 45.54 million hectares which account for 23.25 per cent of the gross cropped area and 37.08 per cent of the area sown to food-grains. Rice production contributes 42.30 per cent of the total food-grain production in the country, continues to play a vital role in the national food basket and livelihood security system. India is one of the leading exporters of rice, particularly basmati rice. The state of West Bengal ranks first in area and production of rice. Punjab has the highest productivity in the country. The major rice growing states are West Bengal, Uttar Pradesh, Andhra Pradesh, Punjab, Tamil Nadu, Orissa, Bihar & Chhattisgarh. These states contribute about 72% of the total area and 76 % of the total rice production in the country. There is a wide variation in the productivity at State level. The states namely Andhra Pradesh, Goa, Haryana, Karnataka, Kerala, Manipur, Punjab, Tamil Nadu, Tripura, West Bengal having productivity above the national average.

India was one of the first country to start academic studies on hybrid rice. The Indian Council of Agricultural Research established a goal-oriented network project on hybrid rice in 1989, and this has received further support from the United Nations Development Programme (UNDP) and (Food and Agriculture Organization) FAO since 1991. The project is now being operated as a well-organized national research network with 12 centers across the country and the Directorate of Rice Research in Hyderabad acting as coordinator (**Paroda and Siddiq, 1996**). The situation for the development and commercialization of hybrid rice in India is very

encouraging. Between 1990 and 1996, more than 700 experimental hybrids were developed and evaluated, and the yields of over 100 combinations exceeded that of the best traditional variety by more than 1 tone/ha. Several hybrid varieties have been released for commercial cultivation. The yield advantage of these hybrids over check varieties ranged from 16.2 to 44.2 per-cent. At present hybrid rice is reported to be grown in approximately two lakh hectares. Area under hybrid rice will further increase after heterotic hybrids suitable to high productivity areas of Punjab, Haryana, coastal region of Andhra Pradesh and for shallow low land areas are identified and an effective transfer of technology is taken up vigorously in the target states **(Viraktamat, 2010)**.

In fact, the hybrid rice research was initiated in 1964 **(Yuan, 1966)**. The genetic tools essential for breeding hybrid rice varieties are as the male sterile line (A-line), maintainer line (B-line) and restorer line (R-line) were developed during 1973 **(Yuan and Virmani, 1988)**. The breeding methodology involves the three approaches (a) Three line method or CMS system which is possible and has been found to be most effective genetic tool for developing hybrids, (b) Two line method or TGMS, PGMS and PTGMS system which is governed by environment and (c) One line system or apomictic system which would enable farmers to use their own seed for the successive crops without experiencing genetic segregation. However, the evolution and wide application of these latter two innovative approaches are not likely to occur in future. Among these, three line approach is being widely adopted in India and had fruitfully resulted in the development of more than thirty five varieties of rice hybrids. The CGMS is essentially CMS with a provision of fertility restoration by nuclear gene(s). Hence, it is also referred to as CMS system. The role of cytoplasm in causing male sterility in rice was reported long back in fifties and the first usable cytoplasmic male sterility-fertility restoration system in rice was developed by substituting genes of *japonica* variety-Taichung 65 into the cytoplasm of the *indica* variety Chinsurah Boro II **(Sampath and Mohanty, 1954)**. However, this could not be exploited for commercial hybrid seed production probably due to strict self-pollinating nature of the crop. The first commercially usable CMS line was developed in China during 1973 from spontaneous male sterile plant isolated in a population of the wild rice *Oryza sativa* of *spontanea* on Hainan Island. Discovery of the source, designated as wild abortive (WA) type is considered a landmark in the history of hybrid rice.

The success of hybrid rice is based on the important findings of some key genes. The wild abortive cytoplasmic male sterility (WA - cms) was found in a spontaneous

mutant of wild rice *Oryza sativa* f. *spontanea* in 1970 (Cheng and Yuan, 1980). Its maintainer gene was present in most of the Chinese varieties and restorer gene in most IRRI varieties. These findings led to the development of the first batch of three line hybrids of rice. The WA – cms has become the most employed system in development of rice hybrids (**Virmani and Edward, 1983; Yuan, 1998**).

The important tool for hybrid seed production is Environment-sensitive genic male sterility system (EGMS), controlled by nuclear gene expression, which is influenced by environmental factors such as temperature, day-length, or both. This male sterility system was first observed in pepper by Martin and Crawford in 1951 and subsequently in different crops. Advantages of the EGMS system, in that there is no need of a maintainer line for seed multiplication, thus making seed production simpler and more cost-effective. Since fertile line can be used as a pollen parent (PP); the frequency of heterotic hybrids is higher among two-line hybrids than among three-line hybrids, thereby increasing hybrid breeding efficiency. Negative effects of sterility-inducing cytoplasm are not encountered. The EGMS trait is governed by major genes, thus enabling their easy transfer to any genetic background and thus increasing diversity among the female (EGMS) parents which helps in reducing potential genetic vulnerability among the hybrids. Since there is no need for restorer genes in the male parents of two-line hybrids, this system is ideal for developing indica/japonica hybrids because most japonica lines do not possess restorer genes.

The discovery in 1973 of Nongken 58 S, a PGMS/TGMS japonica rice line (**Shi, 1981; 1985**), provided the first genetic source for the development of two-line system in hybrid rice. The major feature of such PGMS/TGMS lines is that, under longer day length and higher temperatures they show complete pollen sterility and they can be used for hybrid seed production, while under shorter day length and moderate temperatures they show almost normal fertility, this period can be used for their multiplication. In 1987, China initiated a collaborative research project involving the exploitation of PGMS/TGMS lines to develop two-line system rice hybrids.

Line × Tester cross is a technique which covers all the above features efficiently. This technique was developed by Kempthorne in 1957. It is one of the best approaches for screening the germplasm on the basis of GCA and SCA variances, and effects. The total number of crosses to be made is equal to the product of the number of lines and the number of

testers included in the study. These progenies resulting from line x tester mating, along with or without the parents, can be tested in a replicated trial using suitable field design (**Comstock and Robinson, 1948**).

Heterosis is expressed in three ways, depending on the criteria used to compare the performance of a hybrid (**Gupta, 2000**). These three ways are mid-parents heterosis (the performance of a hybrid compared with the average performance of its parents), better parent heterosis or heterobeltiosis (the performance of a hybrid compared with that of the best parent in the cross) and standard heterosis (the performance of a hybrid compared with high yielding variety in the region). From a practical point of view, standard heterosis is the most important of the two other levels of heterosis because it is aimed at developing desirable hybrids superior to the existing high yielding commercial varieties (**Chaudhary, 1984**).

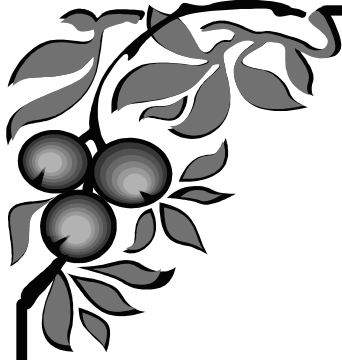
Among the various types of molecular markers available, microsatellite have recently received greater attention, especially for breeding purposes. Microsatellite markers, also known as simple sequence repeats or SSRs (**Litt & Luty, 1989; Weber & May, 1989**) are clusters of short (usually 2 to 6) tandem repeated nucleotide bases distributed throughout the genome. Microsatellite markers are in general co-dominant, multiallelic, and highly polymorphic genetic markers. Microsatellite allele typing requires small amounts of DNA for straightforward PCR and gel electrophoresis analysis (**Rafalski et al., 1996**). Its main disadvantage is the high cost of the initial investment necessary for marker development. However, the number of microsatellite markers available for a model species such as rice is high, and advantage can be taken of this technology in rice genetic research. Hundreds of microsatellite markers have been physically mapped in the rice genome and can potentially be used, as anchor markers, for comparative genetics, trait mapping and gene isolation.

Mapping of TGMS loci has been based mostly on the assumption of a simple genetic control of the trait. For this purpose, plants in a segregating population have been classified in one of two classes, either sterile or fertile (**Subudhi et al., 1997; Dong et al., 2000; Reddy et al., 2000**). It should be noticed that the pollen sterility of segregating populations, derived from crosses between TGMS and fertile lines, does not follow a simple binary distribution. Attempts should be made, therefore, to map TGMS QTL using the percentual of sterile pollen measured in the segregating population, rather than classifying the lines in only two categories (male sterile or fertile).

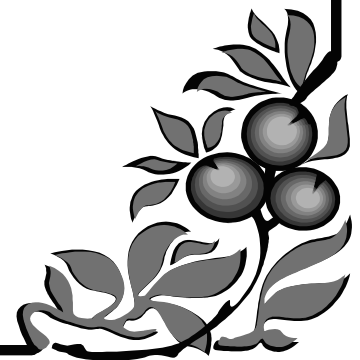
Oryza sativa is composed of two major subspecies, Indica and Japonica (both tropical and temperate) and several ecotypes. Several efforts have been made to assess the genetic diversity within *Oryza sativa* at both phenotypic and molecular levels. To estimate genetic diversity among *Oryza* species, several types of molecular markers, particularly simple sequence repeats (SSR), have been used (Yu *et al.*, 2003; Hashimoto *et al.*, 2004; Garris *et al.*, 2005; Thomson *et al.* 2007; Wen *et al.*, 2009; Ishii *et al.*, 2011, Zhang *et al.*, 2011). Polymorphisms in the SSR region are considered the results of different replications of repeated sequences, resulting in different sizes of the PCR products. However, alleles with different sequences but having the same length may yield ambiguous results of the phylogenetic analysis. Sequencing SSR products can provide clear information on the evolutionary history of these loci (Sunnucks *et al.*, 2000; Provan *et al.*, 2004). Alternatively, single stranded conformation polymorphism (SSCP), a simple and rapid method to determine sequence variation in a large number of samples without expensive direct sequencing, was proposed to use for genotyping and mapping genetic diversity in crop plants (Kuhn *et al.*, 2008). SSCP is a very sensitive technique for the detection of single point mutations between different DNA fragments (Grieu *et al.*, 2004; Muangprom *et al.*, 2005). Recently, SSCP has been used in crop studies, such as marker assisted selection (Borchert and Hohe 2009), comparative genomics (Castelblanco and Fregene 2006), phylogenetics (Rousseau-Gueutin *et al.*, 2009) and fitness effects of crop QTLs (Baack *et al.*, 2008). Furthermore, the recent availability of rice genome sequences provides the opportunity to select genes/sequences distributed in the genome as SSCP markers.

In view of the above, the present investigation was undertaken with the following objectives-

1. Determination of the critical sterility and critical fertility temperature for the different TGMS lines
2. Evaluation and characterization of TGMS lines based on morphological traits and molecular markers
3. To estimates the nature and magnitude of heterosis among set of crosses
4. To estimates the nature of gene action and combining ability of TGMS lines and crosses for yield and other yield contributing traits.



***Review
Of
Literature***



Rice, being the socially and economically most important food crop of the world from ancient times, has been thoroughly studied and well documented. An attempt has been made to review relevant literature related to the following aspects.

2.1 Male sterility system in rice

2.1.1 Cytoplasmic male sterility (CMS)

2.1.2 Environment sensitive genic male sterility (EGMS)

2.1.2.1 Photo-period sensitive genic male sterility (PGMS)

2.1.2.1.1 Development of PGMS lines

2.1.2.1.2 Genetics and breeding behavior of PGMS lines

2.1.2.2 Thermo-sensitive genic male sterility (TGMS)

2.1.2.2.1 Development of TGMS lines

2.1.2.2.2 Genetics and breeding behavior of TGMS lines

2.1.2.2.3 Evaluation of TGMS Lines for critical sterility temperature and critical fertility temperature and other traits.

2.2 Combining ability analysis of parents and crosses

2.2.1 Studies involving pure lines

2.2.2 Studies involving male sterility systems

2.3 Nature and magnitude of heterosis

2.3.1 Heterosis in intra-subspecific crosses

2.3.2 Heterosis in inter-subspecific crosses

2.4 Molecular characterization of TGMS lines with SSR markers

2.1 Male sterility systems in rice

2.1.1 Cytoplasmic male sterility

The role of cytoplasm causing male sterility in rice was first reported in 1954 (Weeraratne, 1954; Sampath and Mohanty, 1954).

Kastuo and Mizushima (1958) observed male sterile plants for the first time in the progeny of the first backcross of *Oryza sativa* f. *spontanea*/O. *sativa* Fujisaka-5.

Shinjo and Omura (1966) from indica × japonica crosses developed the japonica CMS line (nuclear donor, Taichung 65) with indica cytoplasm (Chinsurah Bora II).

Athwal and Virmani (1972) developed a cytoplasmic male sterile line at the International Rice Research Institute by substituting nuclear genes of indica rice variety, Taichung Native 1 with Pankhari 203.

In China, the first cytoplasmic male sterile line was developed in 1973 for the production of commercial F₁ rice hybrids from a sterile plant (wild abortive) occurring naturally in a wild rice population (*Oryza sativa* f. *spontanea* or *O. perennis*) on Hainan Island in 1970.

Virmani et al. (1981) suggested possibility of commercial exploitation of heterosis in rice by the use of cytoplasmic male sterility restoration system and that 45% outcrossing was sufficient to produce bulk quantity of hybrid seed.

Rong-quin (1989) reported CMS line BOA with a very high out-crossing rate (80-85%) and seed yield up-to 4.5 t ha⁻¹ have been obtained.

Pandey et al. (1992) evaluated 13 WA-CMS lines for adaptability and stability for pollen sterility under sub-humid tropic conditions of western UP in northern India. Several stable CMS lines were identified with varying duration and plant type. The CMS lines V 20A, IR 58025A, PMS 1A, PMS 2A, PMS 8A and PMS 10A showed complete pollen sterility and almost 100% spikelet sterility.

Serial and Singh (2001) testcrossed 27 aromatic lines plus 18 non-aromatic and disease resistant types with four cytoplasmic male sterile lines, IR 58025A, IR 62829A, PMS 3A and PMS 10A. Thirteen aromatic and 10 non-aromatic lines were selected based on pooled fertility and crosses were reported to confirm maintenance of sterility and fertility restoring ability. Genotypes were categorized as affective (>80% spikelet fertility), partial restorers (21-79% spikelet fertility) and maintainers (<1% spikelet fertility). The basmati restorers obtained were higher for non-aromatic type. The performance of restorers varied with CMS lines, locations and seasons of testing. The differential ability

to restore fertility in the CMS-WA lines could be because of different nuclear backgrounds of the CMS lines.

Vanaja et al. (2003) identified cytoplasm of vytilla 3, an improved saline tolerant variety of Kerala as a new source for cytoplasmic male sterility in rice (*Oryza sativa* L.) suitable to warm humid tropical climatic conditions experienced in the rice growing tracts of the world. This is a breakthrough in hybrid rice production in this country. The varieties IR 36 and Hraswa (an extra short duration variety of Kerala) are the proposed maintainer lines and a variety of Mattatriveni is the proposed restorer line.

Ingale et al. (2004) evaluated newly developed 20 CMS lines of rice (*Oryza sativa* L.) derived from five different sources of cytoplasm for agronomical traits viz., days to 50 percent flowering, plant height (cm), number of tillers per plant, panicle length (cm), panicle exertion (%) and number of spikelets per panicle and their floral traits viz., angle of floral opening, stigma exertion (%), blooming duration (days), duration of floret opening (min), anthesis duration, pollen sterility (%) and outcrossing (%) with standard check CMS line IR 58025A. Four CMS lines viz. RTN-2A, RTN-11A, RTN-14A and RTN-13A were of early duration and rest of the 16 lines were of medium duration (91-106 days). The CMS lines exhibited significantly maximum values in RTN-19A (plant height), RTN-9A (productive tillers per plant), RTN-17A (panicle length), RTN-18A (per cent panicle exertion), RTN-5A (spikelets per panicle), RTN-10A (blooming duration, outcrossing per cent and angle of floret opening), RTN-11A (stigma exertion per cent), RTN-2A (duration of floret opening), RTN-7A (duration of anthesis), RTN-12A (outcrossing per cent) and RTN-3A, RTN-12A, RTN-16A, RTN-18A (100% pollen sterility) for various characters. Out of 20 CMS lines, 7 CMS lines viz., RTN-10A, RTN-9A, RTN-18A, RTN-7A, RTN-12A, RTN-3A and RTN-16A were found promising for almost all agronomical and floral traits under study. These promising CMS lines showed overall excellent phenotypic acceptability and could be used for development of new hybrid rice combinations.

Ingale et al. (2005) identified 47 effective restorers and 41 maintainers among 188 genotypes for six cytoplasmic male sterile (CMS) lines in a study conducted in Maharashtra, India during 2001-02. The maximum maintainers were observed for COMS-9A line (50) followed by IR 68888A (27%), IR 58025A (9%), PMS-5A (9%) and PMS-10A (19%). The maximum effective restorers were observed for IR-58025A (32%) followed by PMS-5A and

IR-62829A (25%), IR 68888A (24%), COMS-9A (16%) and PMS-10A (14%). The average proportion of maintainers, partial maintainers/partial restorers and effective restorers was 22:54:24 in these crosses. The identified restorers and maintainers could be utilized for future development of new rice hybrids and CMS lines, respectively.

Mahalingam A. *et al.* (2013) studied the genetic parameters and association among the floral traits of CMS lines and identification of parental lines having potential outcrossing ability for hybrid seed production. Among the five CMS lines and fifty one tester lines studied for eleven floral traits, CMS line COMS 23A registered higher mean value for style length, breadth and panicle exertion. The genotype COMS 24A had greater stigma breadth, while COMS 25A had long style with good stigma length and breadth. All the CMS lines had above 99.50% pollen sterility. Analysis of variance revealed significant differences among the CMS lines for all floral traits studied. High heritability coupled with high genetic advance as percent of mean was recorded for five traits viz., anther length, stigma length, style breadth, glume opening angle and stigma exertion rate suggesting the improvement of these characters through simple phenotypic selection. Association analysis of floral traits in the CMS lines revealed that glume opening angle had strong positive and significant association with stigma exertion rate. Stigma length had positive non – significant association with stigma exertion rate. No association between anther size and pollen fertility or spikelet fertility in tester line could be established. Out crossing will be higher in seed production of hybrids resulting from any of the four CMS lines viz., COMS 23A, COMS 25A, CRMS 31A and CRMS 32A since each CMS line had some of the desirable floral trait. COMS 23A and COMS 25A had desirable stigma characters, while CRMS 31A and CRMS 32A had desirable glume opening angle which is highly associated with higher out crossing rate.

Huang *et al.*, (2014) the exploitation of male sterility systems has enabled the commercialization of heterosis in rice, with greatly increased yield and total production of this major staple food crop. Hybrid rice, which was adopted in the 1970s, now covers nearly 13.6 million hectares each year in China alone. Various types of cytoplasmic male sterility (CMS) and environment-conditioned genic male sterility (EGMS) systems have been applied in hybrid rice production. In this paper, recent advances in genetics, biochemistry, and molecular biology are reviewed with an emphasis on major male sterility systems in rice: five CMS systems, i.e., BT-, HL-, WA-, LD- and CW- CMS, and two EGMS systems, i.e., photoperiod- and temperature-sensitive genic male sterility

(P/TGMS). The interaction of chimeric mitochondrial genes with nuclear genes causes CMS, which may be restored by *restorer of fertility (Rf)* genes. The PGMS, on the other hand, is conditioned by a non-coding RNA gene. A survey of the various CMS and EGMS lines used in hybrid rice production over the past three decades shows that the two-line system utilizing EGMS lines is playing a steadily larger role and TGMS lines predominate the current two-line system for hybrid rice production. The findings and experience gained during development and application of, and research on male sterility in rice not only advanced our understanding but also shed light on applications to other crops.

2.1.2 Environment sensitive genic male sterility (EGMS)

Sexuality in higher plants is a delicate and fragile system susceptible to the influence of genes (nuclear and cytoplasmic), chemicals, environment and their interactions. Among environmental factors temperature is a predominate factor influencing the male sterility gene followed by the photoperiod (**Kaul, 1988**).

Shi (1981) reported the Hubei PGMS, many lines with pollen fertility alternations were identified. Some of these were spontaneous mutants, while others were developed by irradiation breeding (**Maruyama et al., 1991**) or selected from the segregating generations following hybridization.

2.1.2.1 Photoperiod sensitive genic male sterility (PGMS)

2.1.2.1.1 Development of PGMS lines

In 1973, Shi Mingshong found a male sterile plant in the field of japonica rice cultivar Nongken 58 in Hubei Province of China (**Shi, 1981**). Plant appeared male sterile when flowered under long day length and male fertile when flowered under short day length conditions. The degree of male sterility was 99-100% at heading under artificial light of more than 14 hours but plants were male fertile under artificial light of less than 13 hrs 45 min (**Lu and Wang, 1988**). This mutant was designated as Hubei-photo sensitive genetic male sterile rice (PHGMS) and this male sterility was controlled by single recessive gene (**Lu and Wang, 1988; Jin et al., 1988**).

2.1.2.1.2 Genetics and breeding behaviour of PGMS lines

Wang et al. (1990) studied the photoperiod conditioned male sterility and its inheritance in rice. The photoperiod sensitive stage proved to be chiefly that of the stamen and pistil primordial and secondly that of panicle branch primordial and meiosis of

microsporocytes. It also appeared that the photoperiod sensitive in variety Nongken 58S is controlled by a single gene.

Yuan *et al.* (1993) reported two kinds of photoperiodic reactions operating simultaneously in growth and development of PGMS lines. The first photoperiodic reaction (FPR) either accelerated or delayed panicle differentiation and heading both and is common to all rice lines. The second photoperiodic reaction (SPR) is novel and determines whether the pollen will be sterile/fertile. SPR is controlled by one/two pairs of gene and is inherited independently of the genes that modify the FRP.

Zhang *et al.* (1994) reported that pollen fertility of PGMS line is regulated by the photo period being sterile at long days and fertile at short days. The critical values of the day length and temperature varied as the PGMS gene(s) were transferred into different genetic backgrounds.

2.1.2.2 Thermosensitive genic male sterility (TGMS)

2.1.2.2.1 Development of TGMS lines

Chinese scientists developed a number of TGMS lines. S1 found in Hunan, showed sterility under high temperature. Its critical temperature is 27°C (**Tan *et al.*, 1990**). The lines 5460S and R 59TS were developed in Fujian and showed fertility under 27/22°C but sterility under 33/28°C (**Sun *et al.*, 1989**).

Maruyama *et al.* (1990, 1991) reported a thermo sensitive genic male sterility (TGMS) mutant induced by 20 kr of gamma rays in Japanese variety Remei. The mutant designated as H 89-1 named as Norin PL 12 exhibited no seed set under 31/24°C, partial fertility under 28/21°C and complete fertility under 25/18°C. Pollen sterility in mutant was not changed by the change in day length. They also studied the inheritance of thermo-sensitive genic male sterility and concluded that thermosensitive genic male sterility of H 89-1 was controlled by a single recessive gene.

Pandey *et al.* (1998) identified a new spontaneous TGMS line, UPRI 95-140 TGMS and studied its agronomic worth and nature of fertility sterility transformation. The line showed semi-dwarf plants with erect flag leaf and good yield potential. Male fertility transformation occurred in it before May 1 in dry season (DS) and August 12 onwards in wet season (WS) under Pantnagar conditions. Spikelet fertility recovery more than 40 per cent in both the season

was indicated. A long period of complete sterility of 104 days suitable for hybrid seed production was observed. The 15 day thermo sensitive temperature (15 dtt) for complete sterility was 27.1°C (DS) to 28.7°C (WS) and for optimum fertility it was 21.8°C (DS) to 26.2°C (WS). Line has shown potential for exploitation in hybrid breeding.

Li and Pandey (1998) developed a new thermosensitive genic male sterile line, UPRI 95-167 TGMS using *in vitro* unpollinated ovary culture technique from F₁ of a cross between UPRI 95-140, a donor of TGMS and UPRI 95-117, an indica rice with good grain quality. This line combined long slender grain similar to male parent and had the feature of spikelet fertility transformation closely correlated to mean maximum daily and mean average daily temperature of the 15 day thermo sensitive stage, five days prior to the heading. It exhibited complete pollen sterility between May 1 and September 5 and 0.5 to 12.3 per cent and 0.5 to 41.5 per cent seed set between September 1 to November 1, 1995 and March 10 to April 17, 1996, respectively in north Indian conditions.

Thiyagarajan K. (2010) Two-line breeding in exploitation of thermo-sensitive genic male sterile system is pursued with the objective of development of hybrids with high heterotic potential. A total of 66 new TGMS lines were developed and screened under sterility favoring environment for tgms gene expression during summer and sterility limiting environment during winter at Paddy Breeding Station, Coimbatore for the past five years. Out of 66 TGMS lines, 15 lines showed stable performance and seven TGMS lines viz., COTGMS 02, COTGMS 07, COTGMS 10, COTGMS 11, COTGMS 12, COTGMS 13 and COTGMS 15 were completely pollen sterile throughout the summer period. These lines had wider sterility expression period with very good floral traits viz., higher pollen sterility per cent, panicle and stigma exertion per cent, wider glume opening favorable for enhanced out-crossing rate and seed set percentage during seed production.

2.1.2.2.2 Genetics and breeding behaviour of TGMS lines

Wu and Yin (1992) studied the genetics and sterility fertility transformation of thermo sensitive genic male sterile lines Annong S1, Hengong S1 and W 615S. All the three lines showed stable fertility transformation and the genes responsible for fertility were thought to be non-allelic. Fertility in Annong S1 and Hengong S1 was controlled by two pairs of recessive genes while that in W 615S, it could not be adequately ascertained. The genetic background influenced the expression of genes responsible for fertility in W 615S and Annong S1.

Zhang *et al.* (1996) grew two TGMS lines Pei-ai 64S and GD2S in green house from January to May 1995. The mean daily temperature was 23°C at fertility alteration sensitivity stage of TGMS lines. After heading pollen fertility and seed setting rates were monitored. They found 50.7 per cent seed set in Pei-ai 64S with mean individual plant yield of 3.5 g and suggested seed multiplication in the green house in winter in successful and is a better way to reproduce TGMS lines at low critical temperature.

Borkakati and Virmani (1994) studied the inheritance of TGMS trait of mutant IR 32364 TGMS and its allelism to tms 2 gene in Norin PL 12 on chromosome 7 and found that the mutants. Both the genes were non allelic to each other and therefore, the new gene in IR 32364 TGMS was tentatively designated as tms 3 (t).

Subudhi *et al.* (1997) mapped a TGMS gene, tms 3 from the F₂ population of the cross between TGMS mutant line IR 32364 TGMS and IR 68. Fertile and sterile bulks were constructed following the classification of the F₂ plants into true breeding sterile, fertile and segregating fertile plants based on F₃ family studies. From the survey of 389 arbitrary primers in the bulk segregant analysis, four RAPD markers were identified, in which three, OPF 18-2600, OPB 19-750 and OPAA 7-550 were linked to tms 3 gene in repulsion phase and one, OPAC 3-640 was linked to tms 3 gene in coupling phase. The tms 3 gene was flanked by OPF 18-2600 and OPAC 3-640 on one side and OPAA 7-550 and OPB 19-750 on the other side. OPAC 3-640 was mapped to the short arm of chromosome 6 using a mapping population available at IRRI. However, no RFLP marker from this region showed linkage to tms 3 gene owing to the lack of polymorphism between the parents.

Reddy *et al.* (1998) reported the pattern of inheritance and allelic relationship among diverse TGMS lines. The results indicated that a single recessive gene governed the TGMS trait. However, in the segregating population, individual TGMS progenies from the same F₂ population showed different fertility levels, indicating that some other gene(s) modify the expression of major gene and affect the degree of fertility. Allelic relationships were examined by studying direct crosses and backcross involving two TGMS lines. Lines developed from Norin PL 12 (tms 2; IR 68292, IR 68294 and IR 68949) at IRRI were allelic to ID 24 and JP 1. Lines IR 32364 (tms 3), JP 8-81S and IC 10 were also allelic. Lines SA 2 and F 61 were non-allelic to all the other lines, and therefore, may possess different genes for TGMS trait.

Reddy *et al.* (2000) tagged the TGMS gene through bulk segregate analysis. TGMS and fertility bulks were made from the F₂ population the cross SA 2 × N 22 (fertile). Operon primers revealed 176 polymorphic products specific to fertile parent N 22 and 19 specific to SA 2. Of these, the 0.7 kb amplicon of OPA 12 and 1.9 kb amplicon of OPSI were specific to the TGMS trait. Bulk segregant analysis of F₂ of the cross ID 24 (TGMS, non-allelic to SA 2) × N 22 revealed a 1.2 kb amplicon of OPB-19 specific to TGMS.

Saxena and Singh (1998) studied the fertility response in 15 TGMS at Pantnagar in Uttar Pradesh. Five lines showed seasonal fertility sterility transformation under high temperature (mean daily average over 29°C) and fertility transformation at lower temperature (mean average 26-28°C).

Dong *et al.* (2000) developed an F₂ population from a cross between a TGMS indica mutant, TGMS-VN 1 and a fertile indica line, CH 1, to identify molecular markers linked to the TGMS gene and subsequently determined its chromosomal location. Among 200 AFLP markers screened, four were linked to the gene in coupling phase. Marker E 5/M 12-600 showed polymorphism in RFLP and was closely linked to the gene on chromosome 2 at a distance of 3.3 cm. The new gene was tentatively designated as tms 4 (t).

Li and Pandey (2001) attempted to locate the tms genes in a new TGMS line, UPRI 95-140 TGMS. The trigenic inheritance of sterility in this line was at variance with monogenic inheritance and tms genes reported earlier. The F₁ of UPRI 95-140 TGMS/UPRI 95-117 crossed as male parent to a set of IR 36 based triples and also with IR 36 as check. The results indicated F₂ segregation of TGMS trait in three-way crosses involving triplo 3 and triplo 7, to be significantly different with the check (IR 36//UPRI 95-140 TGMS/UPRI 95-117). Therefore, two of the major tms genes were located on chromosome 3 and chromosome 7, respectively. As the third and the weakest among all the genes was indistinguishable in the genetic background of UPRI 95-140 TGMS/UPRI 95-117, it was not detected and therefore, could not be located. Results also indicated that the gene on chromosome 3 is non-allelic with all the four known genes because they are located on different chromosomes i.e. 6, 7, 8 and 9, respectively. The gene on the chromosome 7 was located on the same chromosome as the known tms 2, but they were considered non-allelic as the former alone cannot confer complete male sterility while the latter (tms 2) can. Similarly the third tms gene present in UPRI 95-140 TGMS is

impossible to be allelic to known genes, as it is the weakest. The three new genes were therefore designated as tms 4 (t), tms 5 (t) and tms 6 (t), respectively.

Liu *et al.* (2002) studied the breeding and application of *indica* rice thermo sensitive genic male sterile (TGMS) line Kang 201S. It was bred during 1993-2001 in Hunan, China from a cross between Anxiang S (an indica rice TGMS line) and 12119 (a late maturing indica variety) with good grain quality by pedigree selection. It had some characteristics such as low critical sterility temperature, high out crossing rate, good combining ability, fine grain quality, etc. Moreover, in a large-scale demonstration trail, it showed high yielding ability, strong cold resistance and good grain quality. It was examined and approved by the crop variety committee of Hunan Province in March 2001.

Wang *et al.* (2003) genetically analysed and indicated that a single recessive gene named tms 5 controlled sterility of AnnongS1. Earlier tms 5 gene was mapped on chromosome 2 based on F₂ population derived from Annong S1 × Nanjiing 11. A RIL (recombinant inbred line) population from the same cross was developed and used for fine mapping of tms 5 gene. Molecular marker techniques combined with BSA (bulk sergeant analysis) were used. As a result, two AFLP markers (AF 10, AF 8), one RAPD marker (RA 4), one STS marker (C 365-1), one CAPs marker (g 227-1) and 4 SSR marker (RM 279, PM 492, RM 327, R 324) were found to be closely linked to tms 5 gene. The DNA sequences of RFLP marker of C 365 and G 227 were found in GenBank and on the basis of these sequences, many primers were designed to amplify the two parents and their RIL population plants. Finally, the tms 5 gene was mapped between STS marker C 365-1 and CAPs marker G 227-1 at a distance of 1.04 cM from C 365-1 and 2.08 cM from G 227-1.

Rongbai *et al.* (2005) studied that thermosensitive genic male sterility in rice to be useful trait for the exploitation of heterosis, especially of inter specific kind using two-line system. Three pairs of independent recessive (tms) genes with additive effects were involved in TGMS expression in UPRI 95-140 TGMS. Expression of trait in F₂ generation involving 44 different genetic backgrounds indicates monogenic (3F: 1S), digenic (15F: 1S) and trigenic (63F: 1) inheritance with frequencies 18.2, 52.3 and 29.5 per cent, respectively. No single pair of genes was capable of causing complete male sterility. Two pairs of major tms genes in UPRI 95-140 TGMS, non-allelic to any of known tms genes, were located on chromosomes 3 and 7, and tentatively designated as tms 6 (t) and tms 7 (t), respectively.

Rongbai and Pandey (2005) investigated genetics and breeding behaviour of TGMS trait of a new TGMS line UPRI 95-140 TGMS in F₁, F₂ test crosses and F₃ generations of three crosses involving IR 36, UPRI 95-117 and UPRI 95-161 as male parents under sub-tropical conditions of Northern India and reported that two pairs of recessive genes controlled the inheritance of TGMS in duplicate fashion. These genes were expressed as low temperature fertility transformation (26-27.1°C), a long sterility period (>90 days) and reasonable fertility at lower temperature (21.7-23.4°C).

Lee *et al.* (2005) investigated a novel TGMS gene, tms 6 in a spontaneous mutant Sokcho-MS, originated from a Korean japonica variety is complete male sterile at >27°C and fertile at 25-27°C. Genetic analysis and molecular based on SSR, STS and EST markers revealed that a single recessive gene locus involved the control of genic male sterility in Sokcho-MS. By using an F₂ mapping population derived from a cross between Sokcho-MS. By using an F₂ mapping population derived from a cross between Sokcho-MS and a fertile indica variety Neda, the TGMS gene, designated as tms 6, was mapped primarily to the long arm of chromosome 5 of *Oryza sativa* at the interval between markers E 60663 (2.0 cM) and RM 440 (5.8 cM). Subsequently, tms 6 was fine mapped to the interval between markers RM 3351 (0.1 cM) and E 60663 (1.9 cM).

2.1.2.2.3 Evaluation of TGMS Lines for critical sterility temperature and critical fertility temperature and other traits.

Viraktamath & Virmani (2001) Response of thermosensitive genic male sterility in rice to varying temperature situations was studied by using four TGMS lines. In three sets of maximum, minimum and their combined temperatures, it was observed that maximum temperature played a predominant role in influencing sterility/fertility of TGMS lines under the combined regimes. Expression of a TGMS gene was found to be influenced by the genetic background of the recipient lines. Exposure for more than 8 hours of 32 °C was found necessary to induce complete male sterility in indica TGMS lines: IR68945-4-33-4-14 and IR68949-11-5-31 while, more than 4 hours of such exposure was enough to induce sterility in case of their japonica donor Norin PL 12. Sudden interruption with 27 °C even for 2 hours under the sterility inducing regime of 32/24 °C could induce partial fertility in the line IR68945-4-33-4-14. However, the line ID 24 remained completely sterile even with 10 hours of interruption with 27 °C. An interruption with lower temperature of 27 °C for 4 hours for even one day induced partial fertility in IR68945-4-33-4-14. The period of four to eight days

after panicle initiation was the stage most sensitive to temperature variations. Hybrid rice breeders need to develop numerous genetically diverse TGMS lines, which possess critical sterility inducing temperature of 28 °C and are not affected by sudden interruptions with a lower temperature for few hours daily for a couple of days.

Lohithaswa H.C. *et al.*, (2000) thirteen male-sterile clones of rice (*Oryza sativa* L.) variety CTH-1 showing male sterility were isolated from the M₂ generation. They were maintained clonally and evaluated for pollen and spikelet sterility over 58 periodical plantings under high and low temperature regimes at three locations, viz., Hebbal (Bangalore), Honnavile (Shimoga), and Mudigere (Chickmagalore), India. Clones 4, 6, and 10 showed 100% spikelet sterility when the temperature regimes were above 30°C. The sensitive stage, stamen-pistil primordia stage of Clones 4, 6, and 10 was around 23 days, 20 days, and 19 days before heading and critical sterility points were 35.5°C/23.0°C, 35.8°C/23.5°C, and 35.0°C/23.0°C, respectively. Analysis of the F₂ generation of crosses between these three clones and four pollen parents revealed that the sterility of thermosensitive genetic male-sterile clones was under the control of two recessive genes.

Sanchez and Virmani (2005) Correlation analyses between spikelet fertility and maximum and daily mean temperatures at 1-30 days before heading were done to determine the critical stage where temperature influences the sterility/fertility expression of the TGMS lines. The CSP values of the TGMS lines were identified through regression analysis between spikelet fertility and maximum and daily mean temperatures at the critical stage. The data showed that the critical stage for most TGMS lines occurs at the developmental stages between the differentiation of secondary branch primordium and the filling stage of pollen, or approximately 24 to 5 days before heading. Norin PL12, ID24, IR32364 (TGMS), IR72093 and IR72096 were identified to have low CSP (T_{max} <32 °C; T_{mean} <27 °C). The data indicates that evaluating for sterility in the wet season is more effective than in the dry season in identifying TGMS lines with low CSP. TGMS lines developed using this new strategy was found to be more stable in terms of sterility expression. Preliminary evaluation of 2-line hybrids from these low-CSP TGMS lines showed a high frequency of heterotic combinations (77%).

Ramkrishna S. *et al.*, (2006) Thermo sensitive genetic male sterile lines were screened for temperature sensitivity and their morphology and floral biology were studied. All

the six TGMS lines clearly showed a tendency of transformation from sterile to fertile phase, when temperature was decreased in the field as well as under growth chamber conditions. Fertility restoration was more at lower temperature (28/21°C and 24/21°C) for all the six TGMS lines. All the TGMS lines exhibited critical sterility point at temperature more than 30°C. Critical sterility point ranged from 30°C (DRR 1S) to 35.9°C (IR 73827-23S). For all morphological traits except ligule shape variation was observed. Floral biology both in sterile and fertile phase showed varied behavior. In sterile phase the time of anthesis ranged from 9:05AM to 9:40 AM. For duration of anthesis there was clear cut difference among the TGMS lines. DRR 5S took minimum time (165 min) and UPRI 95-140S (270 min) took maximum time. Blooming duration varied from two to four days. Angle of glume opening ranged from 25 to 36°. DRR 5S showed less exerted stigma (25%). High stigma exertion was observed in UPRI 95-167S (70%). Out crossing ranged from 20 to 41%. Maximum out crossing rate was recorded in DRR 1S. In fertile phase time of anthesis ranged from 9:35 AM to 10:30 AM. Duration of anthesis varied from 140 min in DRR 5S to 215 min in UPRI 95-67S. Blooming duration varied from 3-7 days. Angle of glume opening ranged from 15 to 25°. Stigma exertion was highest in UPRI 95-167S (62.4%) and lowest in DRR 5S (20%).

Latha and Thiyagarajan (2010) in this study six TGMS lines viz., TS 6, TS 16, TS 18, TS 29, TS 46 and TS 47 were characterized for their fertility behavior. The lines were screened for pollen and spikelet fertility by raising the plants at fortnightly interval. All the lines had stable sterile phase with 100 per cent pollen sterility for more than 50 consecutive days during high temperature condition (>30/20oC max/min) and they reverted to fertile with more than 60 per cent pollen and spikelet fertility during low temperature condition (<30/20oC max/min). All the lines except TS 29 showed one distinct sterile phase from March to June, whereas TS 29 had two sterile phases from mid February to mid June and from second week of September to first week of November. Since all the lines were completely sterile for more than 30 consecutive days during sterile phase, hybrid seed production utilizing these lines can be taken up by raising the lines in such a way that flowering coincides with sterile phase. All the lines reverted into fertile in two phases. The first fertile phase was short (13-17 days) during July for all the lines except TS 29, for which it was observed during August. The second fertile phase was longer in duration from late November to early February for more than 30 days in all the lines except TS 29, in which it was only for 16 days during December. The maximum pollen and spikelet fertility recorded during this period was 63 to 85 per cent and 58 to 70 per cent, respectively.

Salgotra et al. (2012) Thermo-sensitive genic male sterility (TGMS) is the genic sterile system in plants that affects the fertility/sterility response to temperature in hybrid rice breeding. Eight TGMS lines, DDR 1S, DDR 18S, DDR 19S, DDR 20S, DDR 23S, DDR 27S, DRR 28S and DDR 29, showed satisfactory seed-set percentage at high altitude, but complete sterility at low altitude. Characterization of sterility-sensitive stage and floral traits were determined by the tracking method. At low altitude, with an average air temperature of 35.4 °C, TGMS lines DRR 19S, DRR 20S and DRR 29S displayed a sterility-sensitive stage at 21 days prior to normal heading. The TGMS line DRR 1S required a temperature of 36.6 °C for complete sterility at 17 days prior to normal heading. In the remaining seven lines, the temperature for complete sterility ranged from 33.9 °C to 35.8 °C at low altitude. Angle of opened lemma and palea showed a significantly positive correlation with opening duration of lemma and palea and with size of stigma.

Celine et al., (2014) Two TGMS lines introduced from IRRI namely EC 720903 and EC 720904 were evaluated along with Uma and Jyothi, two popular male fertile rice varieties of Kerala at monthly intervals from January 2012 to December 2013. The male sterile lines showed 100% male sterility during April- May, September - October and January- March months indicating the suitability of the lines as donors of male sterility. Male sterile and fertile lines differed morphologically. TGMS and non TGMS lines differed at molecular level for TGMS gene specific marker RM 3351. The study proves the potential of the TGMS lines to be used as donor parent to transfer male sterility to popular varieties of Kerala by marker assisted back cross breeding

2.2 Combining ability analysis of parents and crosses

Plant breeder is confronted with the problem of choice of parents while breeding for high yielding varieties of the crop plants. Elimination of poor yielding crosses on the basis of per se performance in early generations has been suggested but it was felt that the knowledge of genetic architecture of yield and its components will help to identify the better crosses more efficiently. Combining ability analysis seems to be the most reliable and quickest method of understanding the genetic nature of quantitatively inherited characteristics.

Sprague and Tatum (1942) defined GCA as the average performance of a line hybrid combinations and specific combining ability as those cases in which certain combinations are relatively better or worse than would be expected on the basis of GCA of

their parents. Generally speaking, good combiner parents results in higher frequency of heterotic hybrids than the poor combiner parents. From the genetic viewpoint, GCA measures additive gene effects and SCA measures non-additive gene effects including dominance and epistasis.

Griffing (1956a) and **Carnahan *et al.* (1960)** and many other workers have suggested that the GC include both the additive effects as well as additive $\times$ additive interactions. It has been realized that high yielding lines may not necessarily be able to transmit its superiority to the hybrid (Allard, 1960). Hence an estimate of GCA (worth of parent) and SCA (worth of hybrids) effects may be more reliable test rather than per se performance.

Available literature on the gene action and combining ability for yield and other agronomic characters in rice is presented under the following sub-heads:

2.2.1 Studies involving pure lines

Virmani (1987) stated that almost all kinds of SCA effects can be obtained from any type of parental combinations but the majority of the best SCA combinations involved at least one parent possessing high GCA effects and the other could have high, medium or low GCA for hybrid combinations.

Pandey *et al.* (1992) reported the inheritance of embryo length in Japonica rice indicating significance of both additive and non-additive effects and the additive effects appeared to be more important. The hybrids involving ‘Rikuto Norin 15’ with ‘K 129 and Tsyuake. Japan with ‘Suweon 341’ and ‘Dunghanshali’ with ‘Duansan’ and ‘Suweon 341’ appeared promising for incorporating an adequate embryo length into high yielding rice cultivars.

Ramalimagam *et al.* (1993) observed predominance of non-additive gene action over additive gene action for yield and its major attributes except 1000-grain weight. The best general combiners for grain yield were ACM 27, TNAU 801793 and AS 34011 among lines and ADT among testers. The best specific crosses for seed yield were AS 13744/IR 50, AS 688/ADT 37 and AS 34011/ADT 37. The superior cross combinations resulted from high/high, high/low and low/low. High and positive associations were obtained between per se performance and GCA effects of the parents.

Pandey *et al.* (1994) reported highly significant variance for GCA and SCA from the F_1 and F_2 diallel of parents. Both additive and non-additive genes controlled the inheritance of embryo length with the preponderance of the additive genes. Average dominance overall the loci across all parents was within the range of incomplete dominance. Combining ability analysis confirmed greater importance of additive gene effect in the embryo size. Heritability estimates in narrow sense were very high.

Vivekanandan and Giridharan (1997) derived information on combining ability and genetic variance from data on six yield related traits in eight parents and their 15 progenies derived from a 5 line $\times$ 3 tester cross. The results indicated importance of additive gene action for 100-grain weight and grain length, breadth and thickness. Based on the per se performance and GCA effects, ADT 39 and Improved White Ponni were best parent for the improvement of grain traits besides grain yield. It is suggested that the cross ADT 39/Pusa Basmati 1 and Improved White Ponni/Pusa Basmati 1 are suitable for the recombination breeding, while IR 50/Pusa Basmati 1 may be exploited for heterosis breeding.

Saxena *et al.* (2002) Pooled analysis indicated IR 66159-131-4-3-2, tropical japonica line expressing the maximum GCA effects for RGR and CGR both at 60 days after sowing (DAS) and at maturity, UPRI 1092-30-1-2 for LAI at 45 DAS, IR 65600-32-4-6-1 for LAI at 60 DAS and flowering and NAR, IR 65600-42-5-2 for grain yield and Akihikari for leaf thickness. Fertility environments had significant influence on GCA for most of the character. Parents showing the maximum per se performance were not the best general combiners for different characters except the parent IR 65597-25-1 for plant height, IR 65600-77-52-1 for LAI at flowering and IR 66150-5-23-2 for CGR at maturity. Hybrids exhibiting high SCA effect for grain yield in the specific and pooled environments were the combinations involving genetically diverse parents of TJ/I (IR 65600-42-5-2/UPRI 95-170 and IR 65598-152-15-2/Pant Dhan 4) and TJ/A (IR 65600-77-4-2-1/Dular). The most promising specific crosses for physiological characters were Akihikari/Pant Dhan 4 for LAI, UPR 1230-9-2/Dular for leaf thickness, IR 66159-131-4-3-2/UPRI 95-140 for RGR, IR 66154-52-4-2-2/BSI 10 for CGR and Hinohikari/UPRI 95-140 NAR. Study suggested the importance of combining diverse intersubspecific germplasm for these characters to get better recombinants in the segregating generations and for the best exploitation of higher level of heterosis.

Baskheti (2005) analysed combining ability for grain yield in a line $\times$ tester crosses involving 15 pure lines. Preponderance of non-additive gene effects was observed for all the characters examined. RP 1051-38-85-1 and RP 1125-1552-9-3-4-1 were the best general combiners for grain yield. The best cross combination for grain yield was RP 1015-45-121-1 $\times$ Manhar and was having one of the parents as poor and the other as average combiner. Suitable breeding methods are suggested depending on the gene effects.

Rahimi *et al.* (2010) evaluated fifteen F1 hybrids and their parents were in a randomized complete block design with three replications that generated by half diallel crosses of six diverse rice cultivars. The studied traits were growth period, reproductive period, flag leaf area, plant height, panicle length, number of panicles per plant, number of grains per panicle, 1000-grain weight, grain yield, brown grain length and brown grain width. The significance of specific combining ability (SCA) and general combining ability (GCA) for all studied traits revealed that both additive and non-additive gene effects contributed to the inheritance of the traits.

Patil *et al.*, (2012) Combining ability analysis revealed that, sca variances were higher than gca variances for all the characters except days to maturity which indicated non-additive gene action in the expression of the traits, although non-additive gene action was predominant in the expression of most of the traits. The estimation of gca effects for parents indicating that males Sathi 34-36, GR-5 and female Jaya were the best general combiners for grain yield per plant and some yield contributing traits. The best specific crosses combinations are GR-11 $\times$ Sathi 34-36, Jaya $\times$ GR-103 and Jaya $\times$ IR-28 of either average $\times$ good, good $\times$ poor or good $\times$ average combination.

Thakare *et al.*, (2013) The estimates of gca effects indicated that, among females, IR 68886 A and IR 68897 A and among males IR-44, IR-60, IR-9761, IR-4266-29-4-2-2-2, IR-5638-139-2-2, IR-69701-9-3-1 and IR-71138-49-2-2 were found as good general combiners for grain yield per plant. High sca effects were observed in the crosses, IR 68886 A $\times$ IR-44, IR 68897 A $\times$ IET-15554, IR 68897 A $\times$ IR-56455-206-2, IR 68902 A $\times$ IR-4266-29-4-2-2-2 and IR 68897 A $\times$ IR-62161-184-3-1-3-2. They were found to be the best combinations for grain yield per plant and quality traits. The preponderance of non-additive type of gene actions clearly indicated that selection of superior plants should be use for further improvement.

Dorosti and Monajjem (2014) Line (five) × tester (two) analysis was conducted to determine good parents and the nature of gene action governing yield and its component traits in rice. The line × testers showed significant differences for all traits except for number of spikelets per panicle and hundred grain weight. Parents with low × high general combining ability (GCA) effects resulted in heterotic hybrid combination like the IR62829A/IR57301-158-1R. A low × low GCA gave the best heterotic combination i.e. IR68888A/'Sepidroud'. The study showed GCA to only identify better parental lots and it will be unwise to discard the low GCA types. Predominance of dominance gene action for all the traits studied except for flag leaf width and number of spikelets per panicle suggests heterosis breeding must be strongly pursued for exploitation of the yield advantage over the inbred varieties.

2.2.2 Studies involving male sterility systems

Peng and Virmani (1990) studied combining ability for days to flowering, plant height, grain yield, dry matter and harvest index using line × tester analysis involving seven maintainers and eleven restorer lines. GCA and SCA variances were significant for yield, dry matter, days to flowering and plant height, for harvest index, only SCA variance was significant implying that the first four traits were controlled by both additive and dominant gene action. Harvest index was, however, primarily controlled only by dominant gene action.

Watanesk (1993) evaluated 20 F₁ hybrids developed from four A and five R lines in a line × tester fashion in comparison with traditional cultivars RD 23 and SPR 60. GCA and SCA were analyzed for eight yield related traits. For the A lines, CNTA 10 and CNTA 1 were good general combiners for yield, plant height and panicle length. Among the R lines, SPRLE 75055-352-2-1 and SPRLR 7404-17-1-1-1 were good general combiners for the same three traits plus low unfilled grain percentage.

Ramalingam et al. (1994) evaluated 25 hybrid for their fertility status from five CMS lines and testers was in the range 0.0-97.4 per cent and spikelet fertility 0.0-92.5 per cent. All the five testers acted as completed restorers for WA source and maintainer for *Oryza perennis*.

Ganeshan and Rangaswami (1998) estimated heterosis in 40 hybrids of five WA-based CMS lines with eight cultivar in a line × tester mating design for days to flowering, number of productive tillers, panicle length, spikelet fertility and yield per plant and reported IR 62829-A/ White Ponni as the most promising hybrid followed by MS 37A/Ponni.

Sutaryo (1998) analyzed 25 hybrids alongwith five each of lines and testers. Among lines, IR 53942 was the general combiner for yield, panicle number per hill, filled grain number per panicle, days to maturity, plant height and panicle length. Among testers, IR 68894B showed the highest general combining ability for yield, filled grain per panicle and 1000-grain weight. Result indicated that IR 68894A/IR 54942 was the best specific combination for yield and early maturity.

Dwivedi et al. (1999) studied combining ability for grain yield and ten yield contributing characters in ten rice lines and their 45 hybrids from two dialed cross without reciprocals under three environments (E_1 normal sowing time and high fertility; E_2 NS and optimum fertility and E_3 late sowing and high fertility) at Pantnagar in 1995. The parents were the tropical japonica lines BSI 10, BSI 16, BSI 4116 and B 4122 with wide compatibility gene(s) and the indica lines Govind, Manhar, Pant Dhan 4, Sarjoo 52, Pant Dhan 12 and Narendra 359. Results showed that both GCA and SCA were influenced by environments. The best general combiners were Pant Dhan 4, Sarjoo 52, Narendra 359, B4122 and BSI 10 for grain yield and most of the yield contributing traits. BSI 10 $\times$ Narendra 359, B 4122 $\times$ Pant Dhan 4 and B 4122 $\times$ Narendra 359 were the combination for high yield. BSI 10 $\times$ Govind, BSI 10 $\times$ Pant Dhan 12 and B 4122 $\times$ Pant Dhan 12 showed high heterosis at the intersubspecific level.

Mishra and Seshgiri (2001) studied combining ability and heterosis for various traits from 42 hybrids involving two CMS (IR 58025A and IR 62869A) with 21 testers. The CMS line IR 58025A and the testes R-636-382 and Rajashree were the best general combiners for grain yield and related traits whereas the hybrid IR 58025A $\times$ R-636-405 was best specific combiner for grain yield.

Yograj et al. (2001) estimated combining ability for grain yield and its contributing characters in the line $\times$ tester mating design using three stable WA-CMS lines and 18 well adapted and good yielding male parents as the testers. The results showed importance of both additive and non-additive genetic variance in the expression of all the characters. The relative magnitudes of SCA variances were higher than the GCA variance indicating predominance of non-additive gene action in their inheritance. General combining ability effects indicated CMS IR 46820A to be the best combiner followed by ZS 97A and V20A. All the CMS lines were average combiners for grain yield per plant. Among the testers, IR

36 was the best combiner for most characters including grain yield. Based on SCA effects, the most promising combinations for grain yield were V20A/Sarjoo 52. Most combinations with high SCA effects for grain yield per plant involved average/high general combiners into them and suggested for exploitation in hybrid/recombination breeding.

Gam and Hoan (2002) studied combining ability using ten EGMS lines and 15 high yielding elite lines in line $\times$ tester mating design and found that for yield the male parents had better SCA only for yield, panicle number per hill, grains per panicle and plant height.

Bhave et al. (2003) analyzed the combining ability for yield and yield components (plant height, number of tillers per plant, number of productive tillers per plant, days to flag leaf initiation, days to 50% flowering, days to maturity, panicle length, number of grains per panicle, grain fertility, test weight, grain yield per plant, straw yield and harvest index). The study was conducted using 32 F_1 hybrids, 16 restorer lines and 2 maintainer lines grown in Dapoli, Maharashtra ((India), during the *Kharif* season of 1995. The best general combiners were IR 58025B (restorer line) and KJT 14-7 (maintains line) for most of the characters. IR 62829A, RTN 73, KJT 4 and RTN 2 appeared to be good general combiners for plant height. IR 62829A, RTN 68 and KJT 14-7 appeared to be good general combiners for number of tillers per plant. IR 62829A, RTN 73, KJT 1 and KJT 14 were good general combiners for days to 50 per cent flowering and maturity. IR 58025A and RTN 68 were the best general combiners for panicle characteristics. IR 58025A, RTN 68 and KJT 14-7 were good general combiners for grain yield per plant. IR 58025A $\times$ RTN 68, IR 58025A $\times$ PNL 1 and IR 62829 $\times$ PNL 2, which showed high specific combining ability effects and per se performance, were the best combinations for hybrid breeding.

Li et al. (2003) analyzed the combining ability of nine major agronomic traits by crossing *indica*/japonica lines with *indica* photo-thermosensitive genetic male sterile lines under early and late season condition. The general combining ability (GCA) of plant height, panicles per plant, panicle length, seed setting rate, grain weight per plant in G 2417-1, the GCA of days to heading, spikelets per panicle, filled spikelets per panicle and grain weight per plant in G 3005-4-1 and the GCA of 1000-grain weight in G 2123 were better both in the early and late seasons. On the whole, G 417-1 had better GCA, followed by G 3005-4-1, but G 3005-4-1 had better specific combining ability (SCA), followed by

G 2123. The crosses Hai 35/G 3005-4-1 and Pei ai 64S/G 3005-4-1 obtained high and stable SCA of grain weight in both seasons. There indica/japonica rice lines had higher genetic distance than indica. G 2417-1 and G 3005-4-1 had better exploited value in two-line system of hybrid rice breeding according to the combining ability of nine characters.

Kumar *et al.* (2004) studied 27 hybrids generated by crossing three CMS lines with nine testers along with parents for combining ability for days to 50 per cent flowering, days to maturity, plant height, number of effective tillers per hill, panicle length, sterility per cent, 1000-grain weight and grain yield per plant. Among the male parental lines, Pusa 1040 and PSRM-1-16-48-1 appeared as whole, the best general combiner for combiner for yield and its component traits. The female line IR 58025A was a good combiner for grain yield per plant and its major components. The most promising specific combinations were IR 68886A × Pusa 1040, IR 58025A × Gautam and IR 68886A × PSRM-1-16-48-1.

Zhang (2004) analyzed the combining ability of four PGMS lines and 13 restoring lines of two line indica hybrid rice for eight characters (number of days from sowing to heading, plant height, length of neck panicle, panicle length, number of filled spikelets per panicle, seed setting rate, 1000-grain weight and grain weight per plant) using a $p \times q$ incomplete diallel cross (NCII) design. The results showed that both general combining ability (GCA) and specific combining ability (SCA) of all the characters were significant at 1 per cent level. The role of GCA was more important than that of SCA for most characters except for number of filled grains and grain yield per plant and the role of GCA for restoring lines was greater than that for sterile lines. Sterile line 3087S, Shuguang 6125 and restoring line indica 10038, C 418, Lunhui 422 and Nanjing 16 showed greater GCA and SCA variances for main agronomic characters.

Panwar (2005) conducted an experiment in Kota, Rajasthan, India, during 2001 *Kharif* season on lines IET 13846, IET 15391 and IET 11819 as females, testers PUsa Basmti-1, Taraori Basmati, Kasturi, Basmati, Mahi Sugandha, Pakistani Basmati, IR 64, Ratna, Suraksha and Narendra used as males and 30 hybrids obtained from the line × tester mating design. Data were recorded for days to 50 per cent flowering, days to maturity, plant height, productive tillers per plant, filled grains per panicle, spikelet fertility and biological yield per plant. The mean squares due to lines and testers were significant indicating the prevalence of additive variance for all the characters. The estimates of

general combining ability effects of lines and testers showed that IET 13846, Kasturi Basmati 370, Pusa Basmati-1, Taraori Basmati and IR 64 were good general combiners for grain yield per plant. A total of 14 crosses exhibited positive significant specific combining ability (SCA) effects for grain yield per plant, where eight crosses involved one parent with high general combining ability (GCA) effects and the other having either high or low combining ability effect indicating additive and non-additive genetic interaction. The per se performance, GCA effects of parents, heterobeltiosis and economic heterosis in five superior cross combinations that exhibited positive significant SCA effects indicate that the high performance hybrids need to be ones with high SCA effects and vice-versa.

Satish and Seetharamaiah (2005) studied 15 rice hybrids generated from six parents (ARC 5780, BPT 1768, MTU 2067, NLR 33641, BPT 5204 and BPT 4358) in Bapatta, India during the *rabi* season of 1999-2000 alongwith parents for combining ability for number of productive tillers per hill, total chlorophyll (%), grain yield per plot, protein content (%), number of grains per panicle, leaf nitrogen (%) at flowering stage, volume expansion ratio, L/B ratio, harvest index, test weight, amylase content leaf area index (LAI) and kernel weight. Among the parents BPT 5204, MTU 2067, NLR 33641 were found to be good general combiners for grain yield and leaf nitrogen percentage. BPT 4358 was best general combiner for amylase content. The most promising specific combinations were ARC 5780 × BPT 1768, ARC 5780 × NLR 33641, BPT 5204 × BPT 1768, BPT 5204 × MTU 2067, BPT 5204 × BPT 4258 and NLR 33641 × BPT 4358 for grain yield.

Abhinav Sao and Motiramani N.K. (2006) Among the CMS lines studied, DRR 22A was a good general combiner for plant height, CMS line IR 62829A was a good general combiner for the total number of spikelets per panicle and grain yield per plant, CMS line IR 67684A was also good general combiner for total number of productive tiller per plant, total number of filled spikelet per panicle, spikelet fertility percentage and pollen sterility percentage whereas CMS line IR 68899A was rated as good general combiner for total number of chaffy spikelet per panicle. Tester Swarna was found best general combiner for grain yield per plant. Eight cross combinations exhibited high SCA (Specific Combining Ability) effects for grain yield per plant. These crosses were found to involve at least one parent with high GCA (General Combining Ability) effect and other parents having either high or low GCA effect indicating the involvement of additive as well as non-additive gene action operating in these crosses.

Kumar *et al.* (2006) reported that CMS line IR6888A for earliness and IR 58025A for yield and related traits like days to 50% flowering, plant height, productive tillers per plant, panicle length, number of fertile grains /panicle and 1000 grain weight. Four testers namely Pusa 1040, PSRm-1-16-48-11, RAU 1411-4 and RAU 1414-10 were rated as good general combiners for yield.

Kumar *et al.* (2007) revealed that predominance of additive gene action for the traits like plant height, days to 50% flowering, days to maturity, dry matter harvest index, 100 grain weight and grain length. However, both additive and non additive gene effects was equal importance for grain yield/plant, biological yield/plant, grain length:breath ratio,. Predominance of non-additive gene action was recorded for length of panicle and grain length. Both additive and non additive gene effects were played role in inheritance of yield and its component traits.

Zhu *et al.* (2009) analyzed ten male sterile lines for the study of combining ability for yield and related traits. They found that the number of panicles, plant height, panicle length, grain number of panicle, full grain per panicle was mainly affected by the additive effects. The weight of 1000 grain weight and yield on single plant were affected by the additive effects and non-additive effects together and additive effects were greater than non- additive effect.

Saidaiah (2010) evaluated one hundred and fifteen crosses from five CMS lines and 23 restorers along with parents in line $\times$ tester design for grain yield and yield components. Predominance of non additive gene action was observed for all the characters, suggesting the development of hybrids in rice. The line APMS and PUSA 5A, IBL 57, SG27-77, SG 26-120 and KMR 3 were good general combiners for grain yield and contributing traits. IR 43, IR 55 and IR 60 were good general combiners for dwarf plant types.

Akter *et al.* (2010) The relative contribution of line, tester and combinations of line $\times$ tester interaction of ten characters were calculated and found that panicle weight contributed the highest (69.53%) followed by thousand grain weight (63.62%), yield per plant (54.76%), panicle per meter square 51.52% in their hybrids. IR73328A was identified as good general combiner for shorter plant height (-6.09) and panicle per square meter (16.92). BRRI10A and IR78355A produced significant positive GCA for yield per

plant which could be regarded as good general combiner for higher grain yield. Six hybrid combinations were found with significant and positive SCA effect of which the highest value was obtained from BRRI3A/BRRI14R (2.46) followed by IR73328A/BRRI13R (2.23) for yield per plant.

Bagheri and Jelodar (2010) studied combining ability and heterosis on 12 F1 hybrids along with seven rice genotypes (three cytoplasmic male sterile lines and four restorer varieties) to know the pattern of inheritance of some morphological traits for selecting superior genotypes. Variances of SCA were higher than the GCA variances for traits except for plant height which indicated predominance of non-additive gene action in the inheritance of the traits. The highest heterosis (106.60%) was observed in cross IR68899A x Poya followed by other eight crosses for yield and most of its related traits. The proportional contribution of testers was observed to be higher than that of the interactions of line x tester that revealed the higher estimates of GCA variance that is additive gene action among the testers used. Within CMS parents, IR62829A and among male parents, IR50 and Poya were observed to be good general combiners for most of the characters studied. The cross combinations IR62829A x Mosa-tarom, IR68899A x Poya, IR58025A x IR50 and IR58025A x Poya were observed to be good specific cross combinations for grain yield and most of its related traits due to highly significant SCA and heterotic effects.

Tiwari et al., (2011) the best cross combination in order of merit grain yield and other yield components were IR58025A x IR48749-53-2-2-2R, NMS4A x IR633-76-1R, IR58025A x IR54853-43-1-3R, IR58025A x IR19058-107-1R and PMS10A x IR54853-43-1-3R. Considering the heterosis more than 60% as well as significant *sca* effect for major components, the NMS4A x IR633-76-1R, IR58025A x IR19058-107-1R and IR58025A x IR32419-28-3-1-3R were most promising combinations and need to be tested on large scale. Besides these, some other crosses viz., NMS4A x IR52256-9-2-2-1R, NMS4A x IET 9352 and IR58025A x IET201102, which expressed more than 50% heterosis along with desirable significant *sca* effects for more than six important yield components, may be considered for commercial exploitation.

Gulzar et al. (2012) Heterosis and combining ability estimates were worked out through Line x Tester analysis of 36 hybrids developed by crossing 18 lines (Males) with

two cytoplasmic male sterile (CMS) lines (Females) to know the genetic architecture of various agro-morphological traits in rice for development of hybrids under temperate conditions. The estimates of GCA effects indicated male parent 'K-08-61-2' was good general combiner for grain yield per plant and other yield contributing traits and among the female parent 'SKAU 11A' was observed to be good general combiner. Cross combinations 'SKAU 7A' x 'K-08-61-2', 'SKAU 7A' x 'SR-2', 'SKAU 11A' x 'K-08-60-2', 'SKAU 11A' x 'K-08-59-3' and 'SKAU 11A' x 'SKAU-389' were found to be good specific combinations for grain yield per plant and other desirable traits and needs to be tested on large scale.

Malik and Singh (2013) Combining ability study on grain yield and component traits from a line x tester analysis. Days to 50% flowering, plant height, panicle length, panicle number per plant, tiller per plant, panicle weight, 1000 grain weight, spikelet number per panicle, grain number per panicle, % spikelet fertility and harvest index. Amongst parents PUSA 6A (T3) in testers and UPR 3456-4-2-2 (L14) in lines are good combiners for grain yield and other yield related component traits based on their GCA effects. Top hybrids expressing highest SCA effects for grain yield were obtained with general combiners involved into different parental combinations of UPR 3403-4- 1-1 x UPRI 95-167 (L1 x T1), UPRI 2008-62 x UPRI 95-17A (L20 x T2) and UPR 3428-4-1-1 x UPRI 95-17A(L11 x T2). Two lines viz. UPR 3456-4-2-2 (L14) and UPR 3434-1-1-2 (L12) were identified as good general combiners based on their mean performance and GCA effects for yield and its various traits.

Raju *et al.*, (2014) Five CMS lines were crossed with 13 R lines and the resultant 65 hybrids along with the parents and five checks viz.,KRH-2, DRRH-2, PA-6201 Jaya, and IR- 64 were evaluated for combining ability (Line x Tester design) at three locations viz., Kunaram (Karimnagar District),Warangal and Kampasagar (Nalgonda District) of Telangana .Among the lines APMS 6 A and among the testers JGL 8292, JGL 8605, JGL 17211, JGL 13515 and JGL 3844 are proved to be good combiners for majority of the characters including the yield, by exhibiting the high gca effects. Out of 65 hybrids, the top five hybrids based on sca effects are APMS 8 A x JGL 11110-2 (7.01), APMS 8 A x JGL 11110-1 (5.48), APMS 6 A x JGL 1111-2 (4.77), APMS 8 A x JGL 13515 (4.01) and APMS 8 A x JGL 8605 (3.93), while the mean performance ranged from 27.64 g/plant to 31.22 g/plant. Among the 65 hybrids tested at three locations 21 hybrids, recorded significant positive gca effects for single plant yield.

2.3 Nature and magnitude of heterosis

The first authentic report on plant hybridization can be traced back to 1717 when Thomas Fairchild produced a cross between tobacco varieties, Carnation and Sweet William. The F₁ hybrid was vigorous but sterile, so it was called as Fair Child's Mule.

Shull (1914) defined heterosis as expression of increased vigour, size fruitfulness, speed of development, resistance to diseases and to insects, or to climatic various of any kind manifested by cross bred organism as compared with corresponding inbreds which in the results of unlikeliness in the constitution of uniting parental gametes.

The first report on heterosis in rice was published by Jones (1926) who observed that some F₁ hybrids had more culms and yielded higher than their parents. The plant yield was significant higher than that of high yielding parent.

2.3.1 Heterosis in intervarietal crosses

Pillai (1961) reported that F₁ plants were outstanding in yield and exhibited hybrid vigour. They gave an increased yield of 34 per cent over the mean yield of parents. Heterosis was due to production of greater number of grains and lower spikelet sterility.

Rutger and Shinjyo (1980) indicated significant yield superiority of 11 of the hybrids out of 153 over the best check. Standard heterosis ranged from 16-53 per cent and averaged 41 per cent.

Nijaguna and Mahadevappa (1983) observed high degree of significant heterosis and heterobeltiosis for plant height, 1000-grain weight, straw yield and spikelet fertility in these crosses.

Virmani (1994) reported the prospects of hybrids in the tropics and subtropics using cytoplasmic male sterile (CMS) lines bred at IRRI (IR 58025A, IR 62829A) and India (Punjab male sterile lines). A maintainer breeding programme at IRRI resulted in considerably higher maintainer frequency. Genetically diverse CMS sources identified at IRRI (CMS-ARC and CMS-*Oryza perennis*) and in India (CMS-Kalinga) to overcome potential genetic vulnerability of hybrids, were indicated. No restorer has yet been identified for CMS-*O. perennis* among elite cultivars. TGMS has been identified in Japan and IRRI and is being evaluated. Hybrid rice seed production technology for the tropics is now available.

Pandey *et al.* (1995) evaluated 36 hybrids alongwith parents and the standard variety, Jaya for grain yield per plant and six characters. Most of the crosses manifested significant heterosis for grain yield and panicle number plant, panicle length, grains per panicle, 1000-grain weight and days to maturity. Very few hybrids manifested heterosis for plant height. The range heterosis for seed yield was -6.7 to 258.2 per cent over better parent and -96.1 to 268.2 per cent over mid parent and -96.3 to 301.8 per cent over the standard variety. The best heterotic combinations for grain yield were Prasad/PP 72, Govind/PP 72, Govind/Sita, Prasad/Mahsuri and Govind/Jaya with the respective heterosis estimates for 258.2, 216.8, 177.8, 161.6 and 108.8 per cent for heterobeltiosis, 268.2, 250.8, 178.1, 204.2 and 119.4 per cent for relative heterosis and 301.6, 265.2, 151.6, 285.0 and 108.8 per cent over the best check. Heterosis for grain yield was due to two or more direct yield contributing characters.

Rogbell *et al.* (1998) assessed the nature and extent of relative heterosis and heterobeltiosis under saline stress condition in line $\times$ tester crosses using five saline stress conditions in line $\times$ tester crosses using five saline sensitive lines and seven salt tolerant testers. None of the hybrids exhibited heterosis for all the traits studied. The cross IR 59788-37-1-1-2-1 $\times$ IR 54717-C 10-43-1-2-2-2 and IR 61457-8-3-3-1 $\times$ IR 54717-C 10-43-1-2-2-2 were good combinations among the 35 hybrids evaluated.

Sharma *et al.* (2001) determined heterosis for grain yield and other agronomic characters in half diallel mating design involving seven parents resistant to bacterial blight. Heterosis over mid and better parents ranged between -36.2 to 67.2 per cent and -51.8 to 52.3 per cent, respectively. The cross BBL 307/BJ 1, BBL 307/C 702015, BBL 307/IR 5040, BBL 180/C 702015, C 702015/IR 50400 and RP 633/RP 2151 were identified as the best hybrids based on per se performance, high heterosis and high specific combining ability effects. Significant inbreeding depression for almost all the characters was recorded suggesting the importance of non-additive gene action in the heterotic expression of hybrids. For 100-seed weight, hybrid recorded significantly higher expression in F_2 as compared to F_1 mean. It suggested the epistatic gene action in heterotic expression alongwith dominance.

Agarwal (2003) evaluated rice cultivars viz., Sasyashree, Chambal, Haya, Vikas, Ratna, Kanak and IR 64 including the F_1 and F_2 crossbred progenies during 1998 in Kota, Rajasthan, India. Data were recorded for grains per panicle, grain weight per panicle, 100-

grain weight and crop yield. Heterosis over better parent was highest for grains per panicle, grain weight per panicle and yield per plot in Vikas × Ratna and Sasyashree × Kanak. The highest heterobeltiosis for 100-grain weight was exhibited by Chambal × IR 64. In general, the crosses showing high heterosis also showed high inbreeding depression.

Dela-Rosa *et al.* (2003) evaluated the performance of some TGMS based two-line rice hybrids. A total of 53 experimental two-line hybrids were generated and evaluated in the preliminary yield trial (PYT) during the 2000 wet season (WS) and 2001 dry season (DS). Low frequencies of commercially usable two-line hybrids were generally observed in both trials. Only 5 hybrids combinations out of the 53 hybrids evaluated were found commercially usable with 1.0-1.5 t/ha yield advantage over the highest yielding check variety. All the hybrids evaluated were early maturity with intermediate plant height and tiller number. The commonly observed negative traits associated with the two-line hybrids evaluated are overly exposed panicles, weak straw and low seed yield. These negative traits could be accountable to lack of TGMS lines with improved plant and good combining ability. And since all the hybrids evaluated were from indica by indica crosses, it is also suspected that low frequency of heterotic hybrids could be due to lack of genetic diversity in the parental materials.

Liu *et al.* (2005) studied the heterotic eco types by the analysis of heterosis of the F₁ hybrids which were created in a diallel set of cross between 9 ecological types. The heterotic patterns of japonica rice were also established. The result indicated that japonica ecotype of North-China, Taiwan, Japanese and Korea rice were considered as heterotic ecotypes in this study. The heterotic patterns in this study were japonica ecotype of Korea × Japanese japonica ecotype, japonica ecotype of North West × American rice and Taiwan japonica rice × japonica variety of Japanese. It suggested that North West japonica varieties exist heterotic gene of number of grains and Taiwan japonica varieties, Japanese japonica varieties and Korean japonica varieties exist heterotic genes of number of particle and IRAT varieties have hetrotic gene of grain weight. It was also found that the correlation coefficient between heterosis and the divergence of parents in morphological traits were not significantly different.

Faiz *et al.* (2006) evaluated F₁'s developed by two lines x two tester mating design along with four parental genotypes to estimate heterosis for yield and yield influencing

traits i.e., plant height, number of productive tillers per plant, number of spikelets per panicle, number of filled grains per panicle, sterility % and grain yield. Both the CMS lines reduced the plant height of their respective F₁ hybrids. The highest positive heterosis over better parents was observed for grain yield (41.83 %), number of productive tillers per plant (11.04 %) and number of filled grains per panicle (7.39 %) in the cross of IR69616A x Basmati 385.

Gnanasekaran *et al.*, (2006) Thirty two rice (*Oryza sativa* L.) hybrids involving four TGMS (Thermo Sensitive Gene Male Sterile) lines and eight testers were evaluated for combining ability and heterosis. Among the parents, the TGMS lines viz., GO 98049 and GO 98014, and the testers CB 97033 and IR 72 were the best based on the mean and gea effects. Considering the mean performance, sea effects and standard heterosis, six hybrids viz., GO 98013/CB 97033, GO 98013/CO 47, GO 98014/AOT 45, GO 98014/PMK 2, GO 98049/AOT 39 and GO 98049/CB 97033 were superior for grain yield and kernel quality traits.

Sharma and Malik (2008) conducted an experiment with 70 hybrids evolved from crossing 14 elite *indica* lines and 5 medium duration testers in a line × tester fashion to estimate extend and magnitude of heterosis for grain yield and its components. High heterosis in yield was accompanied by heterosis for one and more of the major yield components. The maximum heterosis for grain yield was 63.27% in case of UPRI 99-73-1 × Jaya.

Malarvizhi *et al.* (2009) reported cross between COMS 14A× IR 55838-B2-2-3-2-3 and COMS 14A× IR 64 showed superiority for number of productive tillers, spikelets fertility, harvest index and IR 68897× IR 64 for number of productive tillers, panicle length, spikelets fertility and harvest index.

Chandiraka *et al.* (2010) explored heterosis in 88 crosses of *indica* rice hybrids. The magnitude of relative heterosis, heterobeltiosis and standard heterosis with (CORH 2 and ADTRH 1) were estimated. Top yielding hybrids viz., GD 98049 × IR63875-196—2-2-1-3, GD 98014 × TKM 11, GD 98049 × TKM 11, GD99017 × TKM 12 exhibited significant standard heterosis over CORH 2 and ADTRH 1. Most of the high yielding hybrids manifested significant positive heterosis for yield contributing traits viz., number of productive tillers per plant, panicle length, and number of filled grains per panicle, spikelets fertility and 1000 grain weight.

Singh et al., (2011) Studied Among a set of 54 hybrids generated using parental lines, 32 showed better parent heterosis (+21.7%) while 19 showed mid-parent heterosis (+15.0%). For the trait yield per plant genetic distance (GD) was negatively correlated with F1 performance ($r = -0.202$), mid-parent heterosis ($r = -0.325^*$; $P < 0.05$), and better parent heterosis ($r = -0.261$), while it was positively correlated with specific combining ability ($r = 0.042$). Based on the grouped genetic distance (GGD), the hybrid combinations were divided into four groups. The GGD showed linear correlation with hybrid performance within the group (GGD = 40–50: $r = -0.07$; GGD = 70–80: $r = 0.32$). This information can be utilized in the development of higher yielding, two-line rice hybrids through selection of intermediately diverse parental lines using GGD.

Tiwari et al., (2011) the results indicated that the manifestation of heterobeltiosis for grain yield was significantly superiority of 43 hybrids ranging from 11.63 to 113.04% and 46 hybrids over standard variety (Sarjoo-52) ranging from 10.48 to 71.56%. Most of the crosses which exhibited superiority over better parent or standard variety for grain yield also showed significant heterosis for number of fertile spikelets and number of spikelets per panicle. These crosses also possessed about 80% pollen viability. Besides grain yield, considerable heterosis was observed for other characters also but its degree varied from character to character.

Ghara et al., (2014) Study of Heritability and heterosis were conducted on F hybrids along with rice genotypes (cytoplasmic male sterile lines and restorer varieties) to know the pattern of inheritance of some morphological traits for selecting superior genotypes. The experiment was carried out according to line x tester mating design. Analysis of variance revealed significant differences among genotypes, crosses, lines, testers and line x tester interactions for tiller number, plant height, days to flowering, panicle length, number of spikelet per panicle, spikelet fertility and grain yield traits. Variances of SCA were higher than the GCA variances for traits which indicated predominance of non-additive gene action in the inheritance of the traits. The highest broad sense heritability (Hb) was observed for Plant height. The highest heterosis was observed in cross Amol A×IR- followed by other eight crosses for yield and most of its related traits.

2.3.2 Heterosis in inter-subspecific crosses

Heu (1967) studied growth duration and hybrid sterility in remote crosses of cultivated rice and reported that certain rice varieties produced fertile F₁ hybrids when crosses with indica and japonica.

Gu et al. (1989) evaluated indica-japonica F₁ hybrids Zaoxiandang/02428 and 3037/02428 and compared with the indica cultivar Shanyo 63. The hybrids gave 7.4 to 41.0 per cent greater yield 49.4 to 52.4 per cent more number of spikelets per unit area and 6.4 to 14.9 per cent better economic coefficient. They also showed higher daily integrals of photo-synthetic rate on both clear and cloudy days.

Liu and Sun (1990) studied panicle structure of indica-japonica rice hybrids and compared with two indica cultivar and their parents. The panicle structure of two indica/japonica hybrids showed a greater total spikelets number. The increase was due to more secondary branches on the panicle and more spikelets on the secondary branches.

Yang (1992) observed that intersubspecific hybrids viz., indica/japonica, indica/javanica and javanica/japonica exhibit very strong heterosis. The F₁ hybrids grow quickly and vigorously throughout the vegetative stage. The yield potential was 20 per cent more than check hybrids.

Yuan (1992) observed the degree of heterosis in different inter and intrasubspecific hybrid groups with the following general trend: indica/japonica > indica/javanica > japonica / javanica > indica / indica > japonica / japonica. The yield potential in terms of per unit area of the best existing indica/indica hybrid developed using CMS system is around 75 kg/ha i.e., about 15 per cent higher than the pure line varieties. The best two line system indica/indica hybrid can outyield the CMS system indica/indica hybrids by 5-10 percent. The indica/japonica hybrids possess highest yield potential both in sink and source. Theoretically, they may have 30 percent yield advantage over the best existing indica/indica hybrids.

Lu et al. (1993) derived information on heterosis from data on yield related traits in five indica-japonica hybrids and their parents. Heterosis for grain yield ranged from 14.0 to 83.3 per cent. All the hybrids showed decreased seed set and therefore, emphasized on improvement of this character as a major breeding objective for indica-japonica hybrid breeding.

Ikehashi *et al.* (1994) reported wide compatibility gene(s) and indica japonica heterosis in temperate countries. They indicated that F_1 sterility in indica japonica hybrids is due to gamete abortion caused by an allelic interaction at a locus in chromosome 6. Some javanicas have a neutral allele at this locus. The gamete abortion neutral allele S_5^n wide compatibility gene (WCG) has been incorporated into japonica types and utilized to develop fertile indica-japonica hybrids. Genetic analysis indicated that a new locus between Est-9 and Rc (red pericarp) in chromosome 7 is responsible for the hybrid sterility in some crosses between javanicas and a variety from the Indian subcontinent. The two S-loci indicate that hybrid sterility in most crosses is caused at one of the loci and that the alleles at the other locus remain neutral. A general system to identify S-loci is being developed. Some WCG lines for indica japonica hybrids have been developed in China and Japan. Two experimental indica-japonica hybrids were tested in Japan. These hybrids were sensitive to cold weather in temperate regions. In BT cytoplasm, which is effective in the japonica background, the Rf gene functions at gamete level, producing 50 percent sterile, lowering the ratio of fertile pollen which aggravates could amage in indica-japonica hybrids. The hybrid sterility shown in male gametes also lowers the pollen fertility in indica-japonica hybrids implying that substantial numbers of male gametes are aborted in the hybrids. Genetic analysis as well as selection is important for developing hybrids with sound pollen. Synchronization of flowering between indicas and javanicas is necessary for seed production.

Zeng *et al.* (1997) reported the order of magnitude of heterosis to be indica/japonica > indica/javanica > indica/indica hybrids. Low seed setting and low grain-straw ratio were the main problem for utilization of indica/japonica hybrids.

Dwivedi *et al.* (1998) observed moderate to high heterosis for yield and 10 related character in 45 crosses involving 6 indica (I) and 4 tropical japonica (J) varieties of rice in three (E1- optimum sowing and high fertility, E2- optimum sowing and optimum fertility and E3- late sowing and high fertility) environments. Trends in magnitude of heterosis for grain yield and plant height were $I \times J > I \times I > J \times I$ and for days to 50 per cent flowering $I \times J > j \times J > I \times I$. Estimates of standard heterosis for grain yield were -64.5 to 146.1 per cent in E1, -70.4 to 82.2 per cent in E2 and -67.2 to 63.8 per cent in E3. Environment E1 seemed to be more favourable for higher heterosis expression than others. Higher heterosis in yield also accompanied heterosis in panicle number, dry matter and spikelet and grain number per panicle. Most estimates for days to flowering were negative. Heterotic $I \times J$

hybrids also recorded maximum heterosis for earliness. Moderate to low standard heterosis for plant height across environment (2.0-13.07%) was recorded. Hybrids were identified in specific environments for direct exploitation in hybrid breeding. Hybrids B 4116 × Sarjoo 52, B 4122 × Plant Dhan 4 and B 4122 × Narendar 359 were more stable than others over the three environments.

Lopez and Virmani (2000) studied heterosis using TGMS lines and reported 1.0-1.6 t/ha higher grain yield in some heterotic hybrids than the inbred check varieties in unreplicated observational yield trials conducted at IRRI. Two of the six two line hybrids yielded significantly higher than the check in a replicated preliminary yield trial.

Saxena (2000) estimated heterosis for 22 morpho-physiological characters in 88 crosses derived from 22 lines representing 14 elite tropical japonica (TJ), five indica (I) and three japonica (J) and four testers—one TJ and Aus (A) each and two I – under optimum (120N: 60P: 40K kg/ha) fertility environments and reported high to moderate degree of heterosis, heterobeltiosis and standard heterosis for grain yield and most of its components under optimum and high fertility environments. Estimates of standard heterosis (%) for grain yield ranged between – 96.71 to 259.98 and -90.79 to 700.24 in optimum fertility and high fertility environments, respectively. The hybrids manifesting maximum heterosis for grain yield were IR 65600-77-4-2/Dular, IR 65598-152-1-5-2/Plant Dhan 4 and Akihikari/Dular under optimum fertility, high fertility and pooled environments, respectively.

Vijayakumar et al. (2002) highlighted the importance of TGMS system for developing heterosis indica/japonica hybrids as existing CMS source among tropical japonica germplasm lacks the restorer genes (Rf). They also reported 25-30 per cent heterosis in indica/tropical japonica hybrids over the best check IR 36.

Kumari et al. (2003) conducted a trial with 42 hybrids involving indica/japonica crosses. The results revealed that the hybrid Palawan/ASD 18 and BPI 76(G)/ASD 18 had the highest standard heterosis for the number of grains per panicle and single plant yield, respectively. This shows that these hybrids can be utilized for further yield improvement.

Wang et al. (2004) analyzed P₁, P₂, F₁, B₁, B₂ and F₂ of indica-japonica hybrid of 3037/02428 by the major gene and minor gene mixed inheritance model. The research showed that apart from single major gene, the wide compatibility (WC) gene inheritance is also

affected by minor genes. Not only major genes effect on fertility but also minor gene's effect should be considered when utilize WC to overcome semisterility between subspecies.

Yang et al. (2004) studied the breeding and application of rice wide compatibility restorer (WCR) line D069, D069 is a new WCR line and an indica-japonica filial generation. It was bred via pedigree selection during 1987-2002 in Sichuan, China by crossing the medium japonica WCR line 02428 as female parent with the multiple cross progenies as male parents (derived from indica restorer line IR 26 × indica). It showed good plant type, strong tillering ability and good restorability. Some of its F₁ hybrids displayed obvious heterosis and were technically identified in Sichuan during 2001-02.

Ji et al. (2005) reported the precise location of the S₅ⁿ gene. In the first-pass mapping, the S₅ⁿ gene was restricted within a 200 kb region by using a population of 242 isogenic lines in combination with high-density markers developed in the S₅ region. In the fine mapping, the S₃ region was further saturated with newly developed markers and more isogenic lines (549 in total) were investigated. Eventually, the S₅ⁿ gene was mapped within a 50 kb region delimited by the left makers J 13 and the right marker J 17. One BAC clone screened from the BAC library of the WC rice variety 02428 covered the whole S₅ region. Sequence analysis of the 50 kb region revealed two candidate genes, coding an aspartyl protease and a hypothetical protein. This result would greatly accelerate both cloning and marker-assisted selection of this important S₅ⁿ gene.

Xin et al., (2011) the study on the genetic basis of heterosis has received significant attention in recent years. In this study, using a set of introgression lines (ILs) and corresponding testcross F₁ populations, we investigated heterotic loci (HL) associated with six yield-related traits in both *Oryza sativa* L. subsp. *indica* and *japonica*. A total of 41 HL were detected on the basis of mid-parent heterosis values with single-point analysis. The F₁ test-cross population showed superiority in most yield-related traits and was characterized by a high frequency of overdominant HL. Thirty-eight of the 41 HL were overdominant, and in the absence of epistasis, three HL were dominant, suggesting that heterotic effects at the single-locus level mainly appeared to be overdominant in rice. Twenty-four HL had a real positive effect, suggesting that they are viable candidates for the improvement of rice yield potential. Compared with the

quantitative trait loci (QTLs) detected in the ILs, only six out of the 41 (14.6%) HL were detected in QTL analysis under the same statistical threshold, indicating that heterosis and trait performance may be conditioned by different sets of loci.

Dan *et al.*, (2014) in this study, five *indica* and seven *japonica* rice varieties were chosen as the parental lines of a complete diallel mating design. Data from six group traits from all of the hybrids and inbred lines were collected. We found that the grain weight per plant, grain number per panicle, tiller per plant, thousand grain weight and plant height, which reflected increased heterosis, were associated with the genetic divergence index (GDI) of the parents. Meanwhile, owing to the reduced seed setting rate, which was also associated with the parents' GDI, the grain production of the hybrids was negatively affected. After analyzing the relationships between the GDI of *indica-japonica* parents and the grain weight per plant of the F₁ hybrids, an ideal GDI value (0.37) for the two *indica-japonica* parents that could provide an optimal balance between the inter-subspecific heterosis and reproductive isolation was proposed. Our findings will help in the strategic design of an inter-subspecific hybrid rice breeding program by identifying the ideal *indica* and *japonica* parents for a hybrid combination to achieve hybrid rice with an optimal yield. This strategic design of an inter-subspecific hybrid rice breeding program will be time saving and cost effective.

2.4 Molecular characterization of TGMS lines with SSR primers

Temnykh *et al.*, (2000) In order to enhance the resolution of an existing genetic map of rice, and to obtain a comprehensive picture of marker utility and genomic distribution of microsatellites in this important grain species, rice DNA sequences containing simple sequence repeats (SSRs) were extracted from several small-insert genomic libraries and from the database. One hundred and eighty eight new microsatellite markers were developed and evaluated for allelic diversity. The new simple sequence length polymorphisms (SSLPs) were incorporated into the existing map previously containing 124 SSR loci. The 312 microsatellite markers reported here provide whole-genome coverage with an average density of one SSLP per 6 cM. In this study, 26 SSLP markers were identified in published sequences of known genes, 65 were developed based on partial cDNA sequences available in GenBank, and 97 were isolated from genomic libraries. Microsatellite markers with different SSR motifs are relatively uniformly distributed along rice chromosomes regardless of whether they were derived from genomic

clones or cDNA sequences. However, the distribution of polymorphism detected by these markers varies between different regions of the genome.

Reddy et al., (2000) the microsatellite RM257, located earlier on chromosome 9, was linked with the TGMS trait in SA2 at a distance of 6.2 cM. RM257 produced a co-dominant polymorphism with 145-bp (sterile) and 132-bp (fertile) products. Both individually and collectively, the markers TS200 and RM257 located on either side of the TGMS locus are very useful for marker-assisted selection.

Wang et al., (2003) Results on the F₂ population, 30 SSR primer pairs flanking the tms5 gene on chromosome 2 were selected to fine map the tms5 gene using the RIL population. As a result, 11 primer pairs (RM492, RM279, RM327, RM324, RM341, RM6, RM263, RM262, RM166, RM208, and OSR28) amplified polymorphism between the two parents. Following identification with a large number of the RIL plants, it was found that RM492, on the short arm of Chromosome 2 near the centromere region, was located apart from the tms5 gene at a distance of 5.4 cM and RM279 was located on the other side of tms5 gene at a distance of 19 cM.

Siwach et al. (2004) identified a total of 229 alleles were detected at the 50 SSR loci and 49 alleles were in fact present in only one of the 24 varieties. The size difference between the smallest and largest allele varied from 1 (RM333) to as high as 82 (RM206). Multiple alleles were observed at 13 loci. Polymorphism information content (PIC) values ranged between 0.0 (RM167) to 0.78 (RM170), with an average of 0.62 per marker

De Alcochete et al., (2005) The objective of this work was to select and use microsatellite markers, to map genomic regions associated with the genetic control of thermosensitive genic male sterility (TGMS) in rice. An F₂ population, derived from the cross between fertile and TGMS indica lines, was used to construct a microsatellite-based genetic map of rice. The TGMS phenotype showed a continuous variation in the segregant population. A low level of segregation distortion was detected in the F₂ (14.65%), whose cause was found to be zygotic selection. There was no evidence suggesting a cause-effect relationship between zygotic selection and the control of TGMS in this cross. A linkage map comprising 1,213.3 cM was constructed based on the segregation data of the F₂ population. Ninety-five out of 116 microsatellite polymorphic markers were assembled into 11 linkage groups, with an average of 12.77 cM between two adjacent marker loci.

The phenotypic and genotypic data allowed for the identification of three new quantitative trait loci (QTL) for thermosensitive genic male sterility in indica rice. Two of the QTL were mapped on chromosomes that, so far, have not been associated with the genetic control of the TGMS trait (chromosomes 1 and 12). The third QTL was mapped on chromosome 7, where a TGMS locus (tms2) has recently been mapped. Allelic tests will have to be developed, in order to clarify if the two regions are the same or not.

Giarrocco et al. (2007) surveyed sixty-nine accessions with 26 simple sequence repeat (SSR) markers revealing the genomic relationship among cultivars. A total of 219 polymorphic bands were detected. Cluster analysis based on pair-wise comparisons of cultivar, genetic similarities resolved the *O. sativa* accessions into two major *O. sativa* groups, *indica* and *japonica*, and the *japonica* group into the subgroups, tropical and temperate. These clusters agree with the pedigree information available on the accessions and almost all Argentina-released cultivars grouped within the *japonica* cluster. Application of DNA polymorphism analysis revealed genomic relationships in Argentine rice germplasm, generating a database useful for cultivar identification, local germplasm conservation, and breeding programs.

Singh et al. (2009) constructed molecular profiling of basmati and non-basmati *indica* rice varieties were made by using the Inter-SSR-PCR and SSR-PCR assays. The amplification products were analyzed using gel electrophoresis. The distance matrix and cluster analysis was made following UPGMA method. All the varieties were clustered in two major groups belonging to irrigated agro eco-system and aerobic agro eco-system. Twelve basmati varieties including traditional and evolved varieties were grouped generally in four clusters. Twenty-two varieties of aerobic rice were clustered in to a single major cluster.

Shah et al. (2010) nearly 30 SSR markers have been used; of which 9 markers were selected from the genomic regions of chromosome 1 and 10 on which *Rf3* and *Rf4* genes were located. Among 30 SSR markers used, 25 SSR loci generated polymorphic patterns and a total of 231 alleles were amplified. The number of alleles per locus ranged from 5 to 17 with a mean of 9.4 alleles per locus. The PIC values for 25 SSR markers varied from 0.74 (RM195, RM10318, and RM258) to 0.92 (RM302).

Singh et al., (2011) a set of morphological traits and SSR markers were used to determine the genetic relationship among 12 elite thermosensitive genic male sterile (TGMS) lines developed at three different research institutions of India. Agro-morphological data

recorded on 20 morphological traits revealed a wide base of genetic variation and a set of four morphological traits could distinguish most of the TGMS lines. Analysis with 30 SSR markers (20 EST-SSRs and 10 genomic SSRs) revealed 27 markers to be polymorphic, amplifying a total of 83 alleles. Each SSR marker amplified 2–6 alleles with an average of 2.76 alleles per marker and a PIC value varying from 0.54 to 0.96. Cluster analysis based on SSR and morphological data clearly differentiated the lines according to their source of origin. Correlation analysis between morphological and molecular data revealed a very poor association ($r = 0.06$), which could be attributed to selection pressure, genetic drift, sampling error and unknown relationship among related lines. The SSR markers discriminated the genotypes distinctly and quantified the genetic diversity precisely among the TGMS lines. Data on the yield per plant indicated that the genotypes grouping under a similar cluster showed same heterotic behaviour as compared to the genotypes from different clusters when crossed to similar pollinators.

Matthayathaworn *et al.*, (2011) in this study, a TGMS line showing non-pollen type thermo-sensitive genic male sterility was used. Crossing between TGMS line (female parent) and normal pollen varieties; CNT1 and PTT1 (male parent) were performed and F1 and F2 populations were developed for each cross. The phenotypic segregation ratio, 3:1 (fertile: sterile pollens) observed in F2 populations of TGMS/CNT1 and TGMS/PTT1 crosses confirmed that the non-pollen type thermo-sensitive genic male sterility is controlled by a single recessive gene. The bulk segregant analysis (BSA) using simple sequence repeat (SSR) markers were deployed to identify the location and genetic effect of this gene. We have generated new set of SSR markers to identify progenies carrying TGMS gene in a cross TGMS/PTT1. The TGMS gene was located on chromosome 2 with 0.0 cM distance from T2 marker. This TGMS gene was located in the same region of previously identified *tmsX* gene. It might be the same allele of this gene of 2 different lines. This marker could be used for marker assisted backcrossing to transfer TGMS gene for developing female parent in two line hybrid rice system.

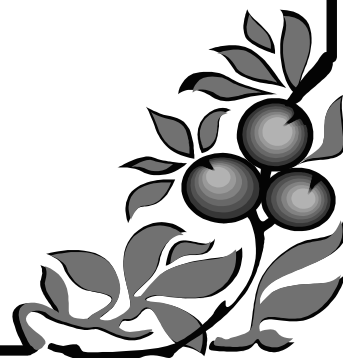
Sajib *et al.* (2012) a total of 24 SSR markers were used across 12 elite aromatic rice genotypes for their characterization and discrimination. Among these 24 markers 9 microsatellite markers were shown polymorphism. The number of alleles per locus ranged from 2 alleles (RM510, RM244, and RM277) to 6 alleles (RM 163), with an average of 3.33 alleles across 9 loci obtained in the study. The polymorphic information content values ranged from 0.14 (RM510) to 0.71 (RM163) in all 9 loci with an average of 0.48.

Mahalingam *et al.* (2013) studied the genetic divergence among 51 restorer and five maintainer lines in terms of grain quality and fertility restorer genes (Rf3, Rf4 and Rf7) using 55 primers of simple sequence repeat (SSR) markers. Among them, 37 SSR markers were found to be polymorphic and the number of amplified fragments ranged from one to five. The highest polymorphic information content (PIC) value (more than 0.60) was observed for eight primers viz., AB 443, RM 3, RM 29, RM 226, RM 228, RM 304, RM 1812 and RM 3873 and average PIC value was 0.444. Cluster analysis using NTSYS generated dendrogram divided all the 56 parental lines into two distinct groups viz., maintainer line (Group I) and restorer line (Group II) at 76% coefficient of similarity. Maintainer line group consisted of five genotypes (COMS 23B, COMS 24B, COMS 25B, CRMS 31B and CRMS 32B) and restorer line group had 51 genotypes. Further, at 80% similarity, all fifty one restorer lines were again grouped into nine clusters. With higher polymorphism revealed by SSR markers, parental lines having the similar genetic background from pedigree information were grouped into different clusters. The combination of pedigree analysis and SSR markers could be a more reliable method to study the diversity and grouping of parental lines of hybrid rice. Hybridization between diverse restorer and CMS groups identified from this study would be expected to yield hybrid combinations with premium grain quality and good fertility restoration.

Celine *et al.*, (2014) Two TGMS lines introduced from IRRI namely EC 720903 and EC 720904 were evaluated along with Uma and Jyothi, two popular male fertile rice varieties of Kerala at monthly intervals from January 2012 to December 2013. The male sterile lines showed 100% male sterility during April- May, September - October and January- March months indicating the suitability of the lines as donors of male sterility. Male sterile and fertile lines differed morphologically. TGMS and non TGMS lines differed at molecular level for TGMS gene specific marker RM 3351. The study proves the potential of the TGMS lines to be used as donor parent to transfer male sterility to popular varieties of Kerala by marker assisted back cross breeding



Materials and Methods



Chapter-3 MATERIALS AND METHODS

The present investigation was carried out at the Norman E. Borlaug Crop Research Center of Govind Ballabh Pant University of Agriculture and Technology, Pantnagar, Uttarakhand (India) during 2013 and 2014. The details of experimental method, materials and statistical techniques followed during course of the investigation have been discussed as below.

3.1 EXPERIMENTAL MATERIAL:

The experimental material was comprised of a total eighteen TGMS lines, four testers and their seventy two F_1 's along with four checks (Table-3.1).

Table-3.1: TGMS lines, testers and their pedigree

Line No.	TGMS Lines	Parentage
TGMS-1	UPRI-99-70-1	UPRI 95-140 TGMS / UPRI 95-141
TGMS-2	UPRI-99-71-1	UPRI 95-140 TGMS / UPRI 95-150 // UPRI 95-162
TGMS-3	UPRI-99-71-2	UPRI 95-140 TGMS / UPRI 95-150 // UPRI 95-162
TGMS-4	UPRI-99-73-1	UPRI 95-140 TGMS / IR 36 // IR Basmati
TGMS-5	UPRI-99-73-2	UPRI 95-140 TGMS / IR 36 // IR Basmati
TGMS-6	UPRI-99-73-3	UPRI 95-140 TGMS / IR 36 // IR Basmati
TGMS-7	UPRI-99-73-4	UPRI 95-140 TGMS / IR 36 // IR Basmati
TGMS-8	UPRI-99-74-3	UPRI 95-140 TGMS / IR BB 21 // IR Basmati
TGMS-9	UPRI-99-79-1	UPRI 95-140 TGMS / UPRI 95-141 // UPRI 95-162
TGMS-10	UPRI-99-60-1	UPRI 95-140 TGMS / UPRI 95-141
TGMS-11	UPRI-99-72-1	UPRI 95-140 TGMS / UPRI 95-150 // UPRI 95-161
TGMS-12	UPRI-99-72-3	UPRI 95-140 TGMS / UPRI 95-150 // UPRI 95-161
TGMS-13	UPRI-99-72-4	UPRI 95-140 TGMS / UPRI 95-150 // UPRI 95-161
TGMS-14	UPRI-99-74-1	UPRI 95-140 TGMS / IRBB-21 // IR Basmati
TGMS-15	UPRI-99-74-4	UPRI 95-140 TGMS / IR BB 21 // IR Basmati
TGMS-16	UPRI-99-75-1	UPRI 95-140 TGMS / IRBB-21 // UPRI-95-150
TGMS-17	UPRI-99-78-1	UPRI 95-140 TGMS / IR 66159-131-4-3-2
TGMS-18	UPRI-97-60-8	UPRI 95-140 TGMS / UPRI 95-140-1
1	Pant Basmati-1	Pusa Basmati / IET 12603 (UPR 908-11-1-1-5)
2	Pant Dhan-4	IR 262 / Remadja
3	Pant Dhan-12	Govind / UPRM 201-1-1
4	UPRI-93-287R	MDC 19340

Checks

- 1 Pant Sankar Dhan-1
- 2 Pant Sankar Dhan-3
- 3 UPRH-128
- 4 UPRH-131

3.2 Observations recorded: observations were recorded on five randomly selected plants from each plot of each replication.

1. Days to 50% flowering

Number of days taken from the date of sowing to the date when 50 per cent plants flowered was recorded on plot basis.

2. Plant height

Plant height was measured in cm from the ground level to the tip of the panicle excluding awns on the main tiller of the plant just before harvesting.

3. Panicle length

It was measured in cm from the neck to the tip of the panicle excluding awn.

4. Number of panicles per plant

The total number of panicles per plant counted at maturity just before harvesting.

5. Spikelet number per panicle

Total number of spikeletes from the panicle of main tiller were counted and recorded.

6. Number of grains per panicle

Total number of filled spikeletes of a panicle of main tiller counted and recorded.

7. Per cent spikelet fertility

It was calculated as the proportion of number of filled spikelets to the total number of spikeletes multiplied by 100.

8. 1000 grain weight

A sample of 1000 seeds fully developed and dried were taken from each plot and weighed in grams on sartorius electronic precision balance.

9. Grain yield per plant

Individual plant was harvested, threshed and dried and weighed for recording grain yield (g) using Sartorius electronic precision balance.

10. Harvest index

Harvest index was calculated as percentage by using the following formula:

$$\text{Harvest Index \%} = \frac{\text{Grain yield per plant (g)}}{\text{Total dry matter yield per plant (g)}} \times 100$$

3.3 Evaluation of TGMS lines

The following sixteen traits were taken for floral and morphological characterization of TGMS lines in order to identify the promising lines which can be included in two line hybrid development.

Table-3.2: Characters observed for morphological evaluation of TGMS lines

S. No.	Characters	Explanation
1	Days to 50% flowering	Number of days taken from the date of sowing to the date when 50 per cent plants flowered was recorded on plot basis.
2	Plant height	Plant height was measured in cm from the ground level to the tip of the panicle excluding awns on the main tiller of the plant just before harvesting.
3	Number of tillers per plant	Total number of tillers per plant were counted.
4	Number of spikelets	Total number spikelets per panicle
5	Panicle type	Type of panicle whether the compact or open
6	Panicle length	It was measured in cm from the neck to the tip of the panicle excluding awn.
7	Apiculous pigmentation	Apiculous is the tip portion of grain, phenotypically observed the color of tip.
8	Stigma color	Color of the stigma observed after seeing the stigma
9	Awning	After emergence of panicle observed that whether awn present or absent

10	Anthesis time	The time at which florets starts to open.
11	Anthesis time duration (min.)	The time duration from opening to closing of florets.
12	Glume angle	At the time of flowering, three well-opened florets of the primary panicle were collected. The angle of the opened florets (angle between lemma and palea) was measured using protractor and expressed in degree.
13	Panicle exertion %	Panicle exertion rate is measured as the ratio of length of panicle that comes out from flag leaf sheath to the total length of panicle and expressed in percentage.
14	Stigma exertion %	The ratio of spikelets with exerted stigma to the total number of spikelets in the panicle was considered as stigma exertion rate and expressed in percentage.
15	Stem color	The color of the lower portion of the stem
16	Pollen sterility	Pollen sterility or fertility was estimated by using 1% iodine potassium iodide (I-KI ₂) solution. Anthers were collected from three randomly selected spikelets (top, middle and bottom) and pollen grains were tweezed out of the anther on glass slide. The fertile (fully stained) and sterile pollen grains (unstained) were counted in five microscopic fields under a light microscope. Pollen sterility was calculated in percentage.

Formulas:

$$\text{Stigma exertion rate (\%)} = \frac{\text{Number of spikelets with exerted stigma}}{\text{Total number of spikelets in the panicle}} \times 100$$

$$\text{Panicle exertion rate (\%)} = \frac{\text{Length of that comes out flag leaf sheath}}{\text{Total length of panicle}} \times 100$$

$$\text{Pollen sterility percent} = \frac{\text{Number of sterile pollen grains}}{\text{Total number of pollen grains (fertile and sterile)}} \times 100$$

3.4 Statistical analysis

The F_1 's crosses planted in randomized block design along with three replications.

3.4.1 Analysis of variance

The steps involved in the analysis of randomized block design were described by Panse and Sukhatme (1961).

Table-3.3: Analysis of variance

Source of variance	d.f.	Mean Square	Expected mean square	F Value
Replication	r-1	MSr	$\sigma_e^2 + g\sigma_r^2$	
Genotypes	t-1	MSt	$\sigma_e^2 + r\sigma_g^2$	MSt/MSe
Error	(r-1)(t-1)	MSe	σ_e^2	
Total	(rt-1)			

Where,

r = number of replications

t = number of genotypes (treatments)

d.f.= degree of freedom

MSr= Mean square of replication

MSt= Mean square of treatment

MSe= Mean square of error

a) Phenotypic variance (σ_p^2) = ($\sigma_g^2 + \sigma_e^2$)

b) Genotypic variance (σ_g^2) = (MSt – MSe)/ r

c) Error variance (σ_e^2) = MSe

3.4.2 Heritability

Heritability in broad sense $h_{[b]}^2$ was calculated for each character as a ratio of genotypic variance to phenotypic variance following Allard (1960).

$$h_{[b]}^2 = \frac{\sigma_{gi}^2}{\sigma_{pi}^2} \times 100$$

Where,

$h_{(b)}^2$ = Heritability in broad sense

σ_{gi}^2 = Genotypic variance of character 'i'

σ_{pi}^2 = Phenotypic variance of character 'i'

Heritability values were categorized according to Johnson *et al.* (1955) as follows:

Low = Less than 0.30, Moderate = 0.30 – 0.60 and High = More than 0.60.

3.4.3 Genetic Advance

The expected genetic advance under selection for different characters under study was estimated by following Allard (1960).

$$G.A. = h_{(b)}^2 \sigma_{pi} \times K$$

Where,

G.A. = Expected genetic advance

$h_{(b)}^2$ = Heritability in broad sense

σ_{pi} = Phenotypic standard deviation of character 'i'

K = Intensity of selection constant, the value of which is 2.06 which is the selection differential at 5% selection intensity as given by Lush, 1949.

Genetic advance as percent of means for each character was calculated as suggested by Johnson, Robinson and Comstock (1955).

$$G.A.(\% \text{ of mean}) = \frac{G.A.}{\text{Mean}} \times 100$$

Where,

G.A.= Expected genetic advance

Mean = General mean of the character

The range of genetic advance in percentage of mean was classified as suggested by Johnson *et al.* (1995).

Low = less than 10%, moderate = 10-20% and high = more than 20%

3.4.4 Combining ability analysis

The combining ability analysis for parental genotypes and their crosses were carried out following Kempthorne, 1957.

3.4.4.1 Line × Tester Analysis

Separate pooled data with lines as rows and columns as testers is tabulated in a two way table, using it we can work out the following sum of squares:

$$\text{S.S. due to lines: } \frac{\text{Sum of squares of lines/rows}}{\text{No. of replications} \times \text{testers}} - \text{C.F. (crosses)}$$

$$\text{S.S. due to testers: } \frac{\text{Sum of squares of testers/columns}}{\text{No. of replications} \times \text{lines}} - \text{C.F. (crosses)}$$

$$\text{S.S. due to line} \times \text{tester:} = \text{S.S. (crosses)} - \text{S.S. (lines)} - \text{S.S. (testers)}$$

Using these S.S. values ANOVA table is made as follows

Table-3.4: ANOVA with Line $\times$ Tester analysis

Source of variation	d.f.	Sum of square (S.S.)	Mean of square (M.S.)	F value
Replications	(r-1)	R.S.S.	RSS/(R-1)	
Lines	(l-1)	L.S.S.	LSS/(l-1)	MSl/MSlt
Testers	(t-1)	T.S.S.	TSS/(t-1)	MSt/MSlt
Line $\times$ Tester	(l-1)(t-1)	L.T.S.S.	LTSS/(l-1)(t-1)	MSlt/MSe
Error	(r-1)(t-1)	E.S.S.	ESS/(r-1)(t-1)	

Now, whole of the information can be compiled in one table excluding the parents as well as follows

Table-3.5: ANOVA for Line $\times$ Tester analysis excluding parents

Source of variation	d.f.	Sum of squares	Mean sum of squares	F values	Expectation
Replications (r)	(r-1)	SSr	SSr/ (r-1)=MSr	MSr/ MSe	
Hybrids (c)	(c-1)	SSc	SSc/ (c-1)=MSc	MSc/ MSe	
Female in Hybrids (fh)	(f-1)	SSfh	SSfh/ (f-1)=MSfh	MSfh/ MSe	$\sigma_f^2 + r\text{Cov (FS)} - 2\text{Cov (HS)} + mr\text{Cov (HS)}$
Males in Hybrids	(m-1)	SSmh	SSmh/ (m-1)=MSmh	MSmh/ MSe	$\sigma_f^2 + r\text{Cov (FH)} - 2\text{Cov (HS)} + fr\text{Cov (HS)}$
Line $\times$ Tester or fh $\times$ mh	(f-1)(m-1)	SSfmh	SSfmh/(f-1)(m-1)=MSfmh	MSfmh/MSe	$\sigma_f^2 + r\text{Cov (FH)} - 2\text{Cov (HS)}$
Error	(c-1)(r-1)	SSe	ESS/(R-1)(T-1)=MSe		σ_e^2

It is to be noted that here M.S. due to lines (MSl) and testers (MSt) are to be tested against the M.S. due to lines $\times$ tester (MSlt). The latter in turn, tested against M.S. due to error (MSe).

3.4.4.2 Estimation of GCA effects

(a). Lines: $g_i = \frac{x_{i..}}{t.r} - \frac{x_{...}}{l tr}$

Where,

- g_i = general combining ability effects of i^{th} line.
- $x_{i..}$ = total of i^{th} line over all the testers and replications.
- $x_{...}$ = sum of all the ($l \times t$) crosses over all the replications.
- l = no. of lines
- t = no. of testers
- r = no. of replications.

(These notations will be used same in proceeding formulae).

(b). Testers: $g_t = \frac{x_{.j}}{l r} - \frac{x_{...}}{l t r}$

Where,

- g_t = general combining ability effects of i^{th} tester.
- $x_{.j}$ = total of j^{th} tester over all the lines and replication,
- $x_{...}$ = sum of all the ($l \times t$) crosses over all the replications.

Further this can be checked by: $\sum g_i = 0$

3.4.4.3 Estimation of SCA effects

The SCA effects for individual lines can be tabulated using the following formula:

$$S_{ij} = \frac{X_{ij.}}{r} - \frac{X_{i..}}{tr} - \frac{X_{.j}}{lr} + \frac{X_{...}}{ltr}$$

Check: $\sum_i S_{ij} = \sum_j S_{ij} = \sum_i \sum_j S_{ij} = 0$

Where,

- S_{ij} = specific combining ability of cross between i^{th} line and j^{th} tester.
- X_{ij} = total of ij^{th} combination over all the replications.

3.4.4.4 Standard Error for combining ability effects

$$\text{S.E. (gca for line)} = \sqrt{\frac{M_e}{r \times t}}$$

$$\text{S.E. (gca for tester)} = \sqrt{\frac{M_e}{r \times l}}$$

$$\text{S.E. (sca effect)} = \sqrt{\frac{M_e}{r}}$$

$$\text{S.E. (g}_i\text{-g}_j\text{) line} = \sqrt{\frac{2M_e}{r \times t}}$$

$$\text{S.E. (g}_i\text{-g}_j\text{) tester} = \sqrt{\frac{2M_e}{r \times l}}$$

$$\text{S.E. (s}_{ij}\text{-s}_{il}) = \sqrt{\frac{2M_e}{r}}$$

Where,

M_e = Mean sum of square of error

3.4.4.5 Genetic Components

Genetic components were calculated following Singh and Chaudhary (1985) as mentioned below:

$$\text{Covariance of half-sib of line} = \text{Cov. H.S. (line)} = \frac{M_l - M_{l \times t}}{rt}$$

$$\text{Covariance of half-sib of tester} = \text{Cov. H.S. (tester)} = \frac{M_l - M_{l \times t}}{rl}$$

$$\text{Covariance of full-sib} = \frac{(M_l - M_e) + (M_t - M_e) + (M_{l \times t} - M_e)}{3r} + \frac{6r \text{Cov. H.S.} - r(l+t) \text{Cov. HS}}{3r}$$

While, *Cov. H.S. (average)* was calculated by the formula given below:

$$\text{Cov. H.S. (average)} = \frac{1}{r(2lt - l - t)} \left[\frac{l-1(M_l) + (t-1)(M_t)}{l+t-2} - M_{l \times t} \right]$$

Assuming no epistasis, variance due to GCA (σ_{gca}^2) and variance due to SCA (σ_{sca}^2) were calculated as followed:

$$\sigma_{\text{gca}}^2 = \text{Cov. H.S.} = \left(\frac{1+F}{4} \right) \sigma_A^2$$

$$\sigma_{\text{sca}}^2 = \text{Cov. F.S.} - 2\text{Cov. H.S.} = \left(\frac{1+F}{2} \right)^2 \sigma_D^2$$

OR

$$2 \sigma_{gca}^2 = \sigma_A^2$$

$$\sigma_{sca}^2 = \sigma_D^2$$

Where,

M_l = Mean sum of square of lines

M_t = Mean sum of square of testers

M_e = Mean sum of square of error.

σ_{gca}^2 = cov H.S = $[1 + F/4] \sigma_A^2$

σ_{sca}^2 = $M_{lt} - M_e/r = [1 + F/4]^2 \sigma_D^2$

Additive and dominance genetic variances (σ_A^2 and σ_D^2) were calculated by taking inbreeding coefficient (F) equal to one i.e. $F = 1$ as cited by Singh and Narayanan (2004).

3.4.4.6 Gene action and degree of dominance

Ratio of $\sigma_{gca}^2 / \sigma_{sca}^2$ less than 1 was taken as preponderance of non-additive type of gene action, greater than 1 as additive and equal to 1 was taken as equal effects of additive and non-additive type of gene action. Similarly ratio of σ_A^2 / σ_D^2 less than 1 was taken as preponderance of non-additive type of gene action, greater than 1, as additive and equal to 1 was taken as equal effects of additive and non-additive type of gene action also. Degree of dominance was calculated by the formula $\sqrt{(\sigma_D^2 / \sigma_A^2)}$ Value of $\sqrt{(\sigma_D^2 / \sigma_A^2)}$ less than 1, was taken as preponderance incomplete dominance, greater than 1, as over-dominance and equal to 1 was taken as equality of complete dominance. All calculations were computed following Singh and Chaudhary (1985).

3.4.4.7 Proportional contribution of lines, testers and line × tester interaction

Contribution of lines, testers and their interaction to the total variance were calculated in accordance with Singh and Chaudhary (1985).

$$\text{Contribution of lines} = \frac{SS(\text{Lines})}{SS(\text{Crosses})} \times 100$$

$$\text{Contribution of Tester} = \frac{SS(\text{Tester})}{SS(\text{Crosses})} \times 100$$

$$\text{Contribution of Line} \times \text{Tester} = \frac{SS(\text{Line} \times \text{Tester})}{SS(\text{Crosses})} \times 100$$

Where, SS (lines), SS (testers), SS (crosses) and SS (line × tester) were the sum of square of lines, testers, crosses and line × tester, respectively.

3.4.4.8 Testing the significance of GCA and SCA effects

Significance of gca and sca effects was tested by 't' test as below:

t value for gca effects in lines = $g_i / \text{SEm } g_i$

t value for gca effects in testers = $g_j / \text{SEm } g_j$

t value for sca effects in crosses = $S_{ij} / \text{SEm } S_{ij}$

Where,

g_i = gca effect of i^{th} line

g_j = gca effect of j^{th} tester

S_{ij} = sca effect of the ij^{th} combination

$\text{SEm } g_i$ = standard error of mean for lines

$\text{SEm } g_j$ = standard error of mean for testers

$\text{SEm } S_{ij}$ = standard error of mean for crosses

Calculated value of 't' was tested against table value of 't' at error degree of freedom at 5 per cent and 1 per cent levels of significance.

3.4.4.9 Estimation of heterosis

Heterosis, expressed as per cent increase or decrease in the performance of F_1 hybrid over the mid-parent (average or relative heterosis), better parent (heterobeltiosis) and check parent (standard heterosis) was computed for each character using the following formula:

$$\text{Relative heterosis} = \frac{\bar{F}_1 - \overline{MP}}{\overline{MP}} \times 100$$

$$\text{Heterobeltiosis} = \frac{\bar{F}_1 - \overline{BP}}{\overline{BP}} \times 100$$

$$\text{Standard heterosis} = \frac{\bar{F}_1 - \overline{CP}}{\overline{CP}} \times 100$$

Where,

$\bar{F}_1$ = Mean performance of F_1 hybrid

$\bar{P}_1$ = Mean performance of parent one

$\bar{P}_2$ = Mean performance of parent two

$\overline{BP}$ = Mean performance of better parent

$\overline{CP}$ = Mean performance of check parent

$\overline{MP}$ = Mean mid-parental value i.e. $(P_1+P_2)/2$

The significance of heterosis was tested with 't' test as given below:

(a) For standard heterosis

$$t = \frac{\overline{F_1} - \overline{CP}}{\sqrt{2Me/r}}$$

Where,

$\overline{F_1}$ = Mean of F_1 hybrid

$\overline{CP}$ = Mean of check parent

Me = Error mean square from ANOVA table and

r = Number of replications

The differences in the magnitudes of relative heterosis, heterosis over male and female parents were tested as per the method proposed by Panse and Sukhatme (1961).

Critical differences (C.D.) were calculated as follows:

For mid parental heterosis:

$$C.D. = \sqrt{\frac{3MSe}{r}} \times \text{'t' value at error d.f.}$$

For heterosis over male and female:

$$C.D. = \sqrt{\frac{2MSe}{r}} \times \text{'t' value at error d.f.}$$

Where,

r = number of replications

MSe = Error mean square

t = table value of 't' at error degree of freedom.

3.5 Molecular marker analysis: The eighteen TGMS lines of rice used for molecular characterization by sixteen SSR markers.

3.5.1 Genomic DNA Extraction

CTAB procedure was used for isolation of DNA (Doyle and Doyle, 1990).

Protocol followed:

1. Two gram of fresh rice seedling leaves were ground to fine powder using liquid nitrogen and a sterilized, pre-chilled mortar and pestle.
2. The powder was transferred as fast as possible into 15ml of prewarmed (60°C) isolation buffer in an oakridge tube.
3. The oakridges were then incubated in water bath at 60°C for 30 minutes. It was mixed gently after every 10 minutes.
4. One volume of chloroform: isoamyl alcohol (24:1) was then added. The tube was capped and shaken gently and thoroughly for 10 minutes by hand to ensure emulsification of the phase.
5. Then it was centrifuged for 10 minutes (5000 rpm, room temperature). Then the (upper) aqueous phase was extracted once again with fresh chloroform: isoamyl alcohol.
6. The final aqueous phase was transferred to a fresh tube using micropipette with a wide bore microtip (that of 1000 µl capacity).
7. 0.6 volume of chilled isopropanol was added, the tube was capped and mixing was done gently but thoroughly by inverting the tube several times. At this stage, the DNA-CTAB complex precipitated as a whitish network. The solution was placed at -20°C for 30 minutes to overnight.
8. Then it was centrifuged (10 min., 5000rpm, 4°C). It was then washed with 70% ethanol, the pellet was gently agitated for few minutes, and collected by centrifugation (10 min., 5000 rpm, 4°C). Residual CTAB was removed by this step.
9. The tubes were inverted and drained on a paper towel for about 1 hour taking care that pellet does not slip down the wall of the tube. It was ensured that it neither contained residual ethanol nor it was too dry. In both cases redissolving might be difficult.
10. An appropriate volume of 1X TE buffer was added (400µl) and the pellet was let dissolve at 4°C without agitation.

Table-3.6: List of SSR marker used for characterization of TGMS lines

S.No.	Primer Name	Gene Bank accession number	Forward Primer	Reverse Primer
1.	RM154	D39059	ACCCTCTCCGCCTCGCCTCCTC	CTCCTCCTCCTGCGACCGCTCC
2.	RM190	X65183b	CTTTGTCTATCTCAAGACAC	TTGCAGATGTTCTTCCTGATG
3.	RM276	CT1715	CTCAACGTTGACACCTCGTG	TCCTCCATCGAGCAGTATCA
4.	RM336	CTT53	CTTACAGAGAAACGGCATCG	GCTGGTTTGTTCAGGTTCCG
5.	RM287	CT838	TTCCCTGTTAAGAGAGAAATC	GTGTATTTGGTGAAAGCAAC
6.	RM279	CT743	GCGGGAGAGGGATCTCCT	GGCTAGGAGTTAACCTCGCG
7.	RM327	CAT99	CTACTCCTCTGTCCCTCCTCTC	CCAGCTAGACACAATCGAGC
8.	RM324	CAT73	CTGATTCCACACACTTGTGC	GATTCCACGTCAGGATCTTC
9.	RM341	CTT77	CAAGAAACCTCAATCCGAGC	CTCCTCCCGATCCCAATC
10.	RM166	X54046	GGTCCTGGGTCAATAATTGGGTTACC	TTGCTGCATGATCCTAAACCGG
11.	RM418	AQ163641	TCGCGTATCGTCATGCATAG	GAGCACATATGCCACGTACG
12.	RM335	CTT50	GTACACACCCACATCGAGAAG	GCTCTATGCGAGTATCCATGG
13.	RM499	AQ574594	TACCAAACACCAACACTGCG	ACCTGCAGTATCCAAGTGACG
14.	RM424	AQ258181	TTTGTGGCTCACCAGTTGAG	TGGCGCATTTCATGTCATC
15.	RM592	AC016779	TCTTTGGTATGAGGAACACC	AGAGATCCGGTTTGTTGTAA
16.	RM204	AF344025	GTGACTGACTTGGTCATAGGG	GCTAGCCATGCTCTCGTACC

3.5.2 Protocol for PCR amplification

Following protocol will be used with minute modification

Cycle	Denaturation		Annealing		Polymerization	
	Temp.	Time	Temp.	Time	Temp.	Time
First cycle	94°C	5 min	—	—	—	—
35 cycles	94°C	1 min	55°C	45 sec.	72°C	2 min
Last cycle	—	—	—	—	72°C	5 min

3.5.3 Purification and quantification of genomic DNA

The quantification of genomic DNA was done by taking the absorbance on Genesys UV spectrophotometer. The optical density was measured at 260 nm and 280 nm. The concentration of the DNA in the sample is related to optical density by the following formula:

$$\text{Conc. of DNA } (\mu\text{g/ml}) = \frac{\text{OD}_{260} \times 50 \times \text{Dilution factor}}{1000}$$

The ratio of $\text{OD}_{260/280}$ is an indication of the amount of RNA or protein contamination in the preparation. A value of 1.8 is optimum for best DNA preparation. A value of the ratio below 1.8 indicates the presence of protein in the preparation and a value above 1.8 indicates that the sample has RNA contamination.

3.5.4 PCR Amplification

PCR reactions were carried out in 9.4 μl PCR master mix and 2 μl genomic DNA. Different SSR primers were taken for amplification.

3.5.5 Master Mix

Tdw=6.8 μl , Buffer = 1 μl , dNTPs = 0.40 μl , Taq Polymerase = 0.20 μl and Primer = 1 μl boh for Forward and Reverse primer.

3.5.6 Electrophoresis of amplified product

PCR products thus obtained were fractionated by using horizontal gel electrophoresis assembly by agarose gel. Agarose gel of 2.5% concentration for SSR primers were prepared by dissolving the calculated amount of agarose in 10 X TBE buffer (Sambrook *et al.*, 1989) and which were examined under the gel-documentation instrument.

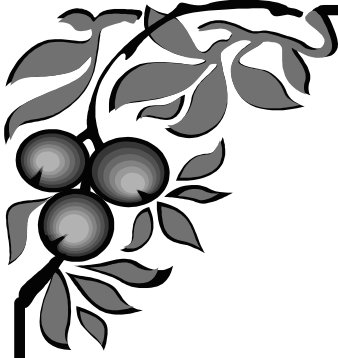
3.5.7 Data analysis

Gels will be documented using Gel Doc system (Bio-Rad). Data were scored as 1 (present) and 0 (absent) for all the alleles of each of the SSR locus. Polymorphism information content was computed as

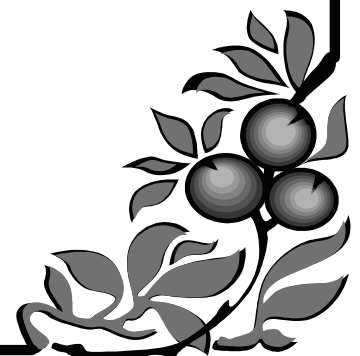
$$1 - \sum_{j=1}^n P_{ij}^2$$

Where,

P_{ij} is the frequency of the j^{th} allele at the i^{th} locus summed over the number of alleles (n). Pair-wise similarity and cluster analysis was done using computer software (NTSYS) was used to perform the similarity matrix analysis using 'UPGMA' with Jaccard's coefficient of similarity.



Results and Discussion



The results of present investigation are presented under the following sub headings:

4.1 Analysis of gene action and combining ability of parents and crosses

4.1.1 Analysis of variance

4.1.2 Estimates of combining ability effects

4.1.3 Contribution of lines, testers and line $\times$ tester to the total variance

4.2 Determining the nature and magnitude of heterosis in the hybrids

4.3 Component of genetic variance

4.3.1 Heritability and genetic advance

4.4 Characterization of TGMS (Thermo-sensitive Genetic Male Sterile Line)

4.4.1 Morphological characterization of TGMS lines

4.4.2 Molecular characterization of TGMS lines

4.1 Analysis of gene action and combining ability of parents and crosses

4.1.1 Analysis of variance

ANOVAs for ten characters viz., days to 50% flowering, plant height, panicle number per plant, panicle length, spikelet number per panicle, grain number per panicle, percent spikelet fertility, 1000 grain weight, harvest index and grain yield per plant are presented in Table 4.1. Results indicated significant difference for all the traits in crosses and line $\times$ tester effect, whereas in line all the traits showed significant difference except plant height, percent spikelet fertility and 1000 grain weight. For tester the significant difference were observed for plant height, panicle length, spikeletes number per panicle, grain number per panicle, percent spikelet fertility and 1000 grain weight. These results are in line with earlier findings of **Ghara *et al.*, (2014)**.

4.1.2 Estimates of combining ability effects

The GCA and SCA effects of the parents and crosses respectively for various characters are given in the Table 4.2 and 4.3. **Sprague and Tatum (1942)** defined GCA as the average performance of a line in a specific hybrid combinations and specific combining ability as those cases in which certain combinations are relatively better or worse than would be expected on the basis of GCA of their parents.

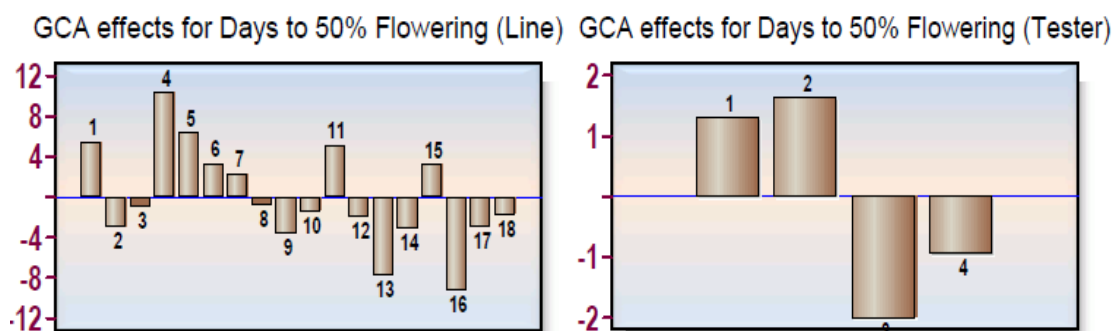
Table 4.1: Line × Tester ANOVA for ten characters

Source of Variations	df	Days to 50% flowering	Plant height (cm)	Panicle number per plant	Panicle length (cm)	Spikelet number per panicle	Grain number per panicle	Percent spikelet fertility	1000 grain weight (g)	Harvest index	Grain yield per plant (g)
Replication	2.00	0.18	9.58	0.06	0.89	25.21	19.27	1.59	0.02	0.31	0.79
Crosses	71.00	144.83**	114.02**	14.48**	7.58**	3567.11**	2020.29**	92.57**	11.80**	113.48**	326.19**
Line Effect	17.00	298.06**	81.72	23.22*	9.54**	5290.46**	2312.41*	109.53	7.30	200.00*	695.73**
Tester Effect	3.00	166.10	1245.42**	12.77	76.12**	29264.29**	14509.41**	297.01*	92.62**	56.40	435.93
Line * Tester Effect	51.00	92.50**	58.24**	11.66*	2.90**	1481.06**	1188.27**	74.90**	8.54**	88.00**	196.55**
Error	142.00	4.07	8.40	0.09	0.53	28.35	22.08	4.93	0.44	1.95	1.86
Total	215.00	50.52	43.29	4.84	2.86	1196.93	681.93	33.84	4.19	38.77	108.96

Generally speaking, good combiner parents results in higher frequency of heterotic hybrids than the poor combiner parents. From the genetic viewpoint, GCA measures additive gene effects and SCA measures non-additive gene effects including dominance and epistasis.

4.1.2.1 Days to 50% flowering

Among the lines, the gca effect were significant and negative for earliness shown by nine lines viz. TGMS-2, TGMS-9, TGMS-10, TGMS-12, TGMS-13, TGMS-14, TGMS-16, TGMS-17 and TGMS-18 while gca effect were significant and positive for lateness shown by seven lines viz. TGMS-1, TGMS-4, TGMS-5, TGMS-6, TGMS-7, TGMS-11 and TGMS-15. Result indicated the line TGMS-16 (-9.21) to be the best combiner for early flowering and it was followed by TGMS-13 (-7.71), TGMS-9 (-3.54) and TGMS-14 (3.13), while for lateness the best combiner was TGMS-4 (10.29); followed by TGMS-5 (6.38), TGMS-1 (5.46) and TGMS-11 (5.13). Among the testers Pant Dhan-12 (-2.00) and UPRI-93-287R (-0.94) showed the significant and negative gca effect for early flowering while Pant Dhan-4 (1.63) and Pant Basmati-1 (1.30) showed the significant and positive gca effect for lateness. **Panwar, (2005)** and **Sharma, (2006)** reported rice genotypes showing negative GCA effects for days to flowering.

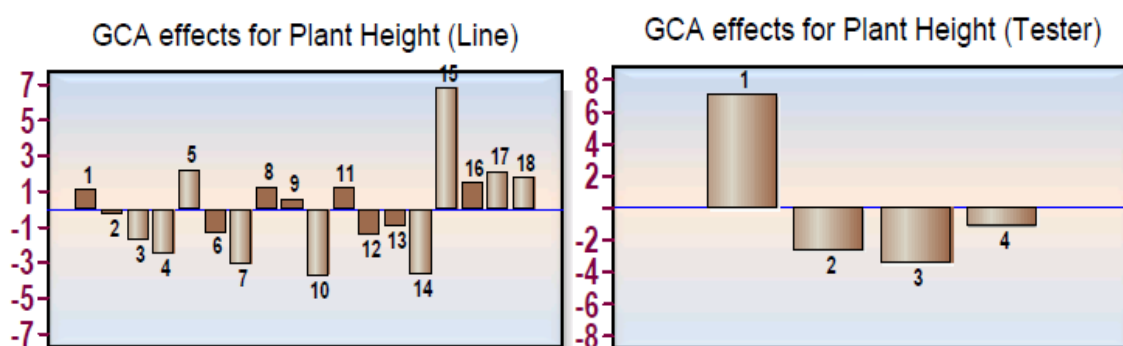


Specific combining ability effects of crosses were significant for 48 crosses, out of which significant and negative crosses were 25. The best specific combinations for early flowering were TGMS-16 × Pant Basmati-1 (-10.55), TGMS-4 × UPRI-93-287R (-8.81), TGMS-14 × Pant Dhan-12 (-8.34), TGMS-18 × Pant Basmati-1 (-8.05) and TGMS-12 × UPRI-93-287R (-7.64). The top crosses which showed high positive and significant sca effect for late flowering were TGMS-8 × Pant Dhan-12 (11.00), TGMS-16 × UPRI-93-287R (9.69), TGMS-1 × UPRI-93-287R (9.36), TGMS-11 × Pant Dhan-4 (8.12) and TGMS-4 × Pant Dhan-4 (7.95). These potential hybrids can

be exploited through heterosis breeding for the identification of superior genotypes with short growth period to fit in wheat-rice cropping pattern. 23 hybrids expressed significant positive SCA effects which mean that these hybrids were not suitable for developing short duration rice varieties. 24 hybrids exhibited non-significant average SCA effects. **Patil *et al.* (2003)**, **Rosamma and Vijakumar (2005)** and **Gnanasekaran *et al.* (2006)** identified promising hybrids based on higher desirable SCA effects for days to flowering.

4.1.2.2 Plant height

Among the lines, GCA effect was significant and negative for plant height. Dwarf plant height is desirable in rice. Five lines viz. TGMS-10 (-3.71), TGMS-14 (-3.66), TGMS-7 (-3.08), TGMS-4 (-2.45) and TGMS-3 (-1.75) had negative and significant GCA effect. For tall plant height the lines TGMS-15 (6.78), TGMS-17 (2.13) and TGMS-18 (1.79) showed significant and positive gca effect. **Gnanasekaran *et al.* (2006)**, **Sharma (2006)** and **Akter *et al.* (2010)** adjudged several parents with desirable GCA effects for dwarf plant height in rice. Results showed that the line TGMS-10 (-3.71) to be the best combiner for dwarf plant type while for tall plant type the best combiner was TGMS-15 (6.78). Among the testers Pant Dhan-12 (-3.40), Pant Dhan-4 (-2.58) and UPRI-93-287R (-1.07) showed significant and negative GCA effect for dwarf plant type while Pant Basmati-1 (7.06) showed significant and positive GCA effect for tall plant type.

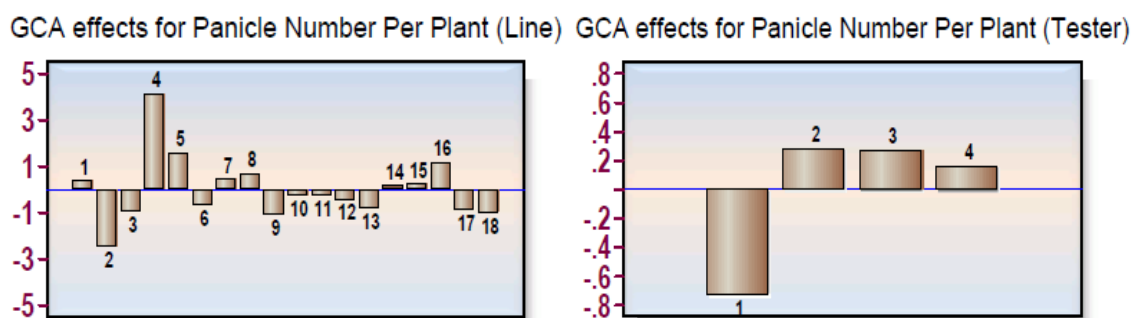


Specific combining ability effects of crosses were significant for 23 crosses, out of which 13 crosses had negative and significant sca effect. The best specific combinations for dwarf plant type were TGMS-16 × Pant Basmati-1 (-10.59), TGMS-11 × Pant Dhan-12 (-7.35), TGMS-1 × Pant Basmati-1 (-6.65), TGMS-11 × Pant Dhan-4 (-6.55) and TGMS-4

× Pant Dhan-4 (-5.44). The top five crosses with high sca effects for tall plant type were TGMS-11 × Pant Basmati-1 (11.09), TGMS-15 × Pant Basmati-1 (7.31), TGMS-16 × Pant Dhan-4 (6.23), TGMS-12 × Pant Basmati-1 (5.68) and TGMS-3 × Pant Dhan-12 (5.30). Ten hybrids expressed significant and positive SCA effects reflecting an increasing trend in plant stature, therefore these were considered undesirable. Rest of the 49 hybrids showed non-significant SCA effects lying in category of average effects. These results were in agreement with those of **Sarker *et al.* (2002)**, **Punitha *et al.* (2004)** and **Sharma (2006)** on the identification of best specific combiner for short plant height.

4.1.2.3 Panicle number per plant

The gca effect were significant and positive for 7 lines, among these the top five best combiner for high number of panicles per plant were TGMS-4 (4.12), TGMS-5 (1.57), TGMS-16 (1.17), TGMS-8 (0.67) and TGMS-7 (0.42). Whereas among the testers, Pant Dhan-4 (0.29), Pant Dhan-12 (0.27) and UPRI-93-287R (0.16) were the best general combiners for high panicle number per plant. **Gnanasekaran *et al.* (2006)** and **Akram *et al.* (2007)** observed promising lines and testers with high GCA effects for number of panicle per plant.



The sca effect were significant and positive for 24 crosses out of which the best five crosses were TGMS-4 × Pant Dhan-4 (4.92), TGMS-7 × UPRI-93-287R (4.74), TGMS-18 × Pant Basmati-1 (3.02), TGMS-14 × Pant Dhan-4 (2.86) and TGMS-17 × UPRI-93-287R (2.64). **Mehla *et al.* (2000)**, **Sharma *et al.* (2005)** and **Sharma (2006)** had reported positive SCA estimates relating to panicle number per plant and recommended importance of high panicle per plant hybrids for the development of hybrid variety(s) or cultivar(s).

Table 4.2: Estimation of general combining ability effects for parents (Line and Testers)

Parents	Days to 50% flowering	Plant height	Panicle number per plant	Panicle length	Spikelet number per panicle	Grain number per panicle	Percent spikelet fertility	1000 grain weight	Harvest index	Grain yield per plant
TGMS-1	5.46**	1.11	0.37**	-1.43**	7.53**	-1.04	-3.28**	0.32	-5.98**	-13.10**
TGMS-2	-2.96**	-0.22	-2.48**	-0.57**	-5.07**	-0.79	1.67**	-0.24	-0.07	-11.60**
TGMS-3	-0.88	-1.75*	-0.93**	-0.95**	7.93**	10.51**	1.46*	0.13	2.70**	-11.00**
TGMS-4	10.29**	-2.45**	4.12**	-0.07	-12.52**	-9.25**	0.35	0.73**	-5.18**	-4.15**
TGMS-5	6.38**	2.22**	1.57**	0.39	-23.32**	-17.94**	0.75	0.69**	-1.91**	-4.75**
TGMS-6	3.29**	-1.27	-0.68**	-0.61**	-0.77	2.96*	1.49*	-0.32	-0.53	-0.25
TGMS-7	2.29**	-3.08**	0.42**	-0.10	-14.82**	-7.70**	2.10**	-1.64**	-0.87*	1.50**
TGMS-8	-0.79	1.24	0.67**	-1.63**	-26.12**	-16.44**	3.70**	-0.09	-0.72	-3.80**
TGMS-9	-3.54**	0.58	-1.08**	-0.19	14.63**	17.21**	1.89**	0.24	-2.14**	1.25**
TGMS-10	-1.38*	-3.71**	-0.23**	0.20	-7.57**	4.90**	5.76**	-0.54**	7.27**	3.30**
TGMS-11	5.13**	1.20	-0.23**	0.92**	7.43**	5.56**	-0.58	-1.04**	-3.29**	0.55
TGMS-12	-1.88**	-1.44	-0.43**	0.93**	9.18**	1.05	-3.55**	0.07	-3.05**	1.20**
TGMS-13	-7.71**	-0.95	-0.78**	-0.11	3.28*	-0.35	-1.89**	-1.15**	6.97**	2.50**
TGMS-14	-3.13**	-3.66**	0.17	1.57**	-31.52**	-22.69**	2.01**	1.28**	2.64**	10.60**
TGMS-15	3.21**	6.78**	0.22*	1.11**	23.28**	11.31**	-4.32**	0.16	-6.07**	-4.35**
TGMS-16	-9.21**	1.48	1.17**	-0.40	-27.12**	-20.00**	1.41*	0.32	4.70**	9.80**
TGMS-17	-2.88**	2.13*	-0.88**	1.14**	47.83**	28.81**	-4.88**	-0.26	3.49**	12.80**
TGMS-18	-1.71**	1.79*	-0.98**	-0.24	27.68**	13.91**	-4.09**	1.33**	2.03**	9.50**
SE (g _i)	0.58	0.84	0.09	0.21	1.54	1.36	0.64	0.19	0.40	0.39
CD 95%	1.15	1.65	0.17	0.42	3.04	2.68	1.27	0.38	0.80	0.78
Pant Basmati-1	1.30**	7.06**	-0.72**	1.66**	-2.44**	-2.45**	-0.33	-1.38**	0.32	-0.53**
Pant Dhan-4	1.63**	-2.58**	0.29**	0.04	33.59**	23.19**	-2.50**	1.78**	1.17**	1.52**
Pant Dhan-12	-2.00**	-3.40**	0.27**	-0.71**	-17.39**	-15.22**	-0.33	-0.25**	-0.23	-3.74**
UPRI-93-287R	-0.94**	-1.07**	0.16**	-0.99**	-13.76**	-5.53**	3.17**	-0.14	-1.27**	2.76**
SE (g _i)	0.27	0.39	0.04	0.10	0.72	0.64	0.30	0.09	0.19	0.18
CD 95%	0.54	0.78	0.08	0.20	1.43	1.26	0.60	0.18	0.38	0.37

*, ** significant at 5 and 1%

Table 4.3: Estimates of specific combining ability effects for crosses

S. No.	Crosses	Days to 50% flowering	Plant height	Panicle number per plant	Panicle length	Spikelet number per panicle	Grain number per panicle	Percent spikelet fertility	1000 grain weight	Harvest index	Grain yield per plant
1.	TGMS-1*Pant Basmati-1	-4.55**	-6.65**	0.48**	-2.10**	-42.26**	-40.60**	-4.05**	-1.27**	-3.38**	-1.77*
2.	TGMS-1*Pant Dhan-4	-0.88	2.17	2.47**	0.23	19.71**	-10.84**	-10.44**	-0.81*	-9.69**	-0.62
3.	TGMS-1*Pant Dhan-12	-3.92**	2.93	-1.53**	-0.24	20.89**	27.17**	3.81**	0.82*	9.53**	3.85**
4.	TGMS-1*UPRI-93-287R	9.36**	1.56	-1.42**	2.11**	1.66	24.28**	10.67**	1.25**	3.54**	-1.45
5.	TGMS-2*Pant Basmati-1	2.87*	-4.30*	1.12**	-0.60	0.74	3.15	1.19	-1.66**	-2.38**	4.73**
6.	TGMS-2*Pant Dhan-4	-2.47*	3.02	-1.28**	0.81	6.91*	1.31	-2.56*	-0.75	-1.52	-3.72**
7.	TGMS-2*Pant Dhan-12	-3.50**	2.76	0.13	-0.01	-9.31**	-12.28**	-2.23	0.84*	-0.76	3.15**
8.	TGMS-2*UPRI-93-287R	3.11**	-1.47	0.03	-0.20	1.66	7.83**	3.61**	1.57**	4.66**	-4.16**
9.	TGMS-3*Pant Basmati-1	0.78	-2.84	-1.23**	1.38**	17.74**	4.25	-5.04**	2.10**	-1.62*	1.34
10.	TGMS-3*Pant Dhan-4	-6.88**	0.42	0.56**	0.41	-13.69**	3.01	6.09**	0.88*	3.26**	1.68*
11.	TGMS-3*Pant Dhan-12	1.41	5.30**	2.38**	-0.77	19.29**	14.02**	-1.20	-2.07**	1.99*	6.54**
12.	TGMS-3*UPRI-93-287R	4.69**	-2.89	-1.71**	-1.02*	-23.35**	-21.27**	0.15	-0.92*	-3.64**	-9.56**
13.	TGMS-4*Pant Basmati-1	5.62**	-0.54	-1.87**	-0.56	6.00	11.40**	3.43**	-2.96**	6.86**	1.48
14.	TGMS-4*Pant Dhan-4	7.95**	-5.44**	4.92**	-0.46	-19.24**	-5.84*	4.92**	3.14**	-4.75**	-2.37**
15.	TGMS-4*Pant Dhan-12	-4.75**	2.30	0.72**	1.57**	4.34	8.77**	3.22*	2.30**	-0.22	-1.51
16.	TGMS-4*UPRI-93-287R	-8.81**	3.67*	-3.77**	-0.55	8.91**	-14.32**	-11.57**	-2.48**	-1.89*	2.39**
17.	TGMS-5*Pant Basmati-1	-2.47*	-4.53**	-0.92**	-0.53	4.79	15.70**	6.61**	-0.31	0.58	-3.52**
18.	TGMS-5*Pant Dhan-4	3.87**	1.45	2.06**	-0.33	-9.84**	-6.55*	0.49	-0.65	-3.54**	6.23**
19.	TGMS-5*Pant Dhan-12	-1.50	2.95	0.07	0.53	0.94	-11.13**	-6.93**	2.32**	1.67*	-4.51**
20.	TGMS-5*UPRI-93-287R	0.11	0.12	-1.21**	0.32	4.11	1.98	-0.16	-1.35**	1.29	1.79*
21.	TGMS-6*Pant Basmati-1	-3.38**	-2.55	-0.88**	0.76	-18.96**	-13.20**	2.04	0.55	-3.30**	-3.22**
22.	TGMS-6*Pant Dhan-4	-1.72	1.15	-1.68**	0.64	-2.39	3.96	2.18	-0.99*	6.68**	10.13**

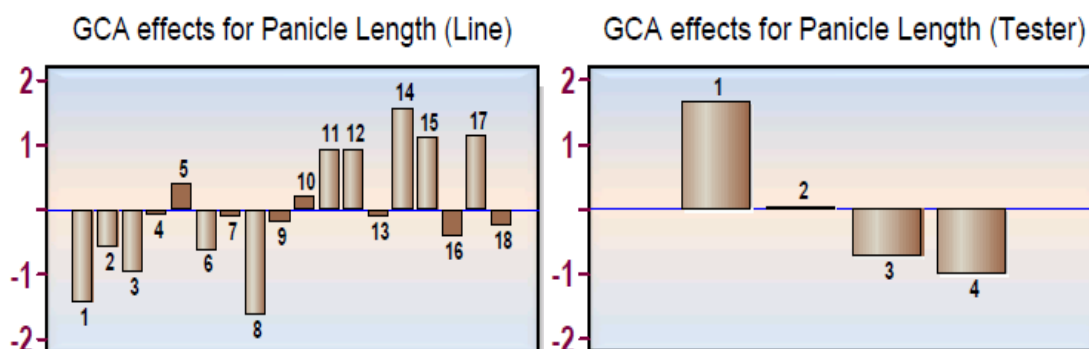
23.	TGMS-6*Pant Dhan-12	-2.42*	1.13	2.53**	-0.98*	-26.41**	-23.23**	0.40	1.92**	2.51**	4.99**
24.	TGMS-6*UPRI-93-287R	7.52**	0.28	0.03	-0.42	47.75**	32.47**	-4.63**	-1.47**	-5.90**	-11.90**
25.	TGMS-7*Pant Basmati-1	4.95**	1.27	-0.97**	0.85*	11.70**	14.45**	2.45	-1.33**	0.12	-0.37
26.	TGMS-7*Pant Dhan-4	-5.72**	-1.81	-2.39**	0.38	-2.94	7.40**	4.20**	0.74	6.61**	2.78**
27.	TGMS-7*Pant Dhan-12	2.25	0.23	-1.38**	-0.72	0.04	2.02	1.92	-0.10	-7.32**	-6.75**
28.	TGMS-7*UPRI-93-287R	-1.48	0.30	4.74**	-0.52	-8.80**	-23.87**	-8.57**	0.69	0.60	4.34**
29.	TGMS-8*Pant Basmati-1	-2.63*	-0.98	-0.03	0.58	-4.80	0.20	2.26	0.54	3.91**	0.53
30.	TGMS-8*Pant Dhan-4	-2.63*	-1.35	1.77**	-0.49	-1.04	-13.04**	-7.34**	-2.31**	-5.93**	-0.32
31.	TGMS-8*Pant Dhan-12	11.00**	-0.85	-2.42**	0.33	1.14	7.17**	3.86*	1.41**	3.05**	4.55**
32.	TGMS-8*UPRI-93-287R	-5.73**	3.18	0.68**	-0.42	4.71	5.68*	1.22	0.36	-1.03	-4.76**
33.	TGMS-9*Pant Basmati-1	4.12**	3.84*	1.73**	0.97*	16.84**	21.35**	2.75*	0.85*	5.00**	21.88**
34.	TGMS-9*Pant Dhan-4	-0.22	-4.68**	-0.09	-1.80**	7.81*	3.71	-1.56	-0.23	-5.66**	-8.77**
35.	TGMS-9*Pant Dhan-12	1.75	4.66**	-0.28	0.97*	-3.81	-5.28	-0.77	-0.56	0.60	-6.91**
36.	TGMS-9*UPRI-93-287R	-5.64**	-3.81*	-1.36**	-0.13	-20.84**	-19.77**	-0.42	-0.05	0.06	-6.20**
37.	TGMS-10*Pant Basmati-1	0.62	0.40	-0.53**	-0.33	-7.56*	-4.35	1.38	-0.87*	-0.37	1.43
38.	TGMS-10*Pant Dhan-4	-0.72	2.64	-0.34	1.12**	42.41**	40.40**	-0.29	-1.00**	2.89**	2.58**
39.	TGMS-10*Pant Dhan-12	1.91	-0.48	1.08**	-0.44	-25.61**	-27.18**	-1.67	0.50	2.29**	-0.56
40.	TGMS-10*UPRI-93-287R	-1.81	-2.57	-0.21	-0.35	-9.24**	-8.87**	0.58	1.37**	-4.81**	-3.46**
41.	TGMS-11*Pant Basmati-1	0.12	11.09**	-0.32	0.35	2.44	1.80	-0.27	-0.25	2.74**	0.58
42.	TGMS-11*Pant Dhan-4	8.12**	-6.55**	-1.34**	-1.18**	17.01**	15.76**	0.41	1.21**	-2.55**	-5.07**
43.	TGMS-11*Pant Dhan-12	-4.25**	-7.35**	-0.33	0.16	-0.61	-5.64*	-2.74*	-1.26**	-7.46**	-4.81**
44.	TGMS-11*UPRI-93-287R	-3.98**	2.80	1.99**	0.67	-18.85**	-11.92**	2.60*	0.29	7.27**	9.30**
45.	TGMS-12*Pant Basmati-1	2.45**	5.68**	0.08	1.30**	-6.91*	-10.90**	-2.60*	-0.48	0.25	0.73
46.	TGMS-12*Pant Dhan-4	2.12	-4.62**	0.06	-1.15**	11.26**	9.85**	0.64	0.42	0.38	-0.52
47.	TGMS-12*Pant Dhan-12	3.08**	-3.74*	-0.33	-0.67	2.04	7.27**	2.64*	-1.19**	-4.17**	-2.45**
48.	TGMS-12*UPRI-93-287R	-7.64**	2.68	0.19	0.52	-6.39*	-6.22*	-0.68	1.26**	3.54**	2.24**

49.	TGMS-13*Pant Basmati-1	-3.38**	-1.38	0.43*	-0.30	-14.41**	-23.90**	-6.17**	-0.50	4.89**	0.03
50.	TGMS-13*Pant Dhan-4	-2.05	1.72	-2.39**	0.37	18.16**	20.85**	2.40	-1.72**	-0.03	-7.42**
51.	TGMS-13*Pant Dhan-12	3.58**	-2.18	1.22**	-0.81	-13.86**	-0.53	6.47**	1.33**	-0.97	9.44**
52.	TGMS-13*UPRI-93-287R	1.86	1.83	0.74**	0.74	10.11**	3.58	-2.70*	0.88*	-3.89**	-2.06*
53.	TGMS-14*Pant Basmati-1	6.37**	4.88**	-1.93**	0.02	1.00	-1.35	-0.88	0.63	-4.84**	-16.27**
54.	TGMS-14*Pant Dhan-4	1.37	1.46	2.86**	-0.62	5.16	20.21**	6.96**	-1.94**	4.19**	21.28**
55.	TGMS-14*Pant Dhan-12	-8.34**	-3.67*	-1.52**	-0.15	4.14	-7.58**	-6.40**	-0.08	-0.88	-9.06**
56.	TGMS-14*UPRI-93-287R	0.61	-2.67	0.59**	0.76	-10.29**	-11.27**	0.32	1.39**	1.52	4.04**
57.	TGMS-15*Pant Basmati-1	3.37**	7.31**	2.62**	-0.14	20.79**	10.05**	-2.85*	2.55**	-13.94**	-6.12**
58.	TGMS-15*Pant Dhan-4	-1.30	1.43	-1.18**	1.35**	-43.04**	-29.19**	3.28*	2.47**	7.26**	7.43**
59.	TGMS-15*Pant Dhan-12	2.33*	-4.29*	-0.37*	0.39	-5.26	-1.99	1.03	-3.60**	5.87**	1.10
60.	TGMS-15*UPRI-93-287R	-4.39**	-4.46**	-1.07**	-1.60**	27.51**	21.13**	-1.46	-1.43**	0.81	-2.40**
61.	TGMS-16*Pant Basmati-1	-10.55**	-10.6**	0.07	-1.35**	-15.00**	-18.45**	-2.97*	-0.07	11.18**	5.74**
62.	TGMS-16*Pant Dhan-4	-3.88**	6.23**	0.26	-0.46	-50.04**	-38.50**	2.21	-0.79*	-3.34**	-13.52**
63.	TGMS-16*Pant Dhan-12	4.75**	1.31	-0.53**	1.59**	35.74**	24.72**	-3.03*	-0.44	-6.56**	-5.46**
64.	TGMS-16*UPRI-93-287R	9.69**	3.04	0.19	0.22	29.30**	32.23**	3.78**	1.29**	-1.27	13.24**
65.	TGMS-17*Pant Basmati-1	3.78**	1.39	-0.88**	-0.13	43.04**	34.35**	-0.72	1.51**	-1.48	0.34
66.	TGMS-17*Pant Dhan-4	2.12	-1.76	-2.88**	0.00	1.41	-14.49**	-5.11**	0.41	-1.57	-8.12**
67.	TGMS-17*Pant Dhan-12	-6.59**	-0.58	1.12**	0.32	-17.21**	-1.28	5.15**	-0.17	2.46**	-2.06*
68.	TGMS-17*UPRI-93-287R	0.69	0.95	2.64**	-0.19	-27.24**	-18.57**	0.67	-1.75**	0.59	9.84**
69.	TGMS-18*Pant Basmati-1	-8.05**	-1.51	3.02**	-0.17	-15.21**	-3.95	3.42**	0.98*	-4.23**	-7.57**
70.	TGMS-18*Pant Dhan-4	2.95*	4.51**	-1.39**	1.16**	12.36**	-8.00**	-6.46**	1.90**	7.31**	-1.63*
71.	TGMS-18*Pant Dhan-12	3.25**	-0.43	-0.57**	-1.06*	13.54**	5.02	-3.54**	-1.98**	-1.62*	10.44**
72.	TGMS-18*UPRI-93-287R	1.86	-2.56	-1.06**	0.07	-10.70**	6.93*	6.59**	-0.90*	-1.45	-1.25
	CD 95% SCA	2.30	3.31	0.34	0.83	6.08	5.36	2.53	0.76	1.60	1.56

*, ** significant at 5 and 1%

4.1.2.4 Panicle length

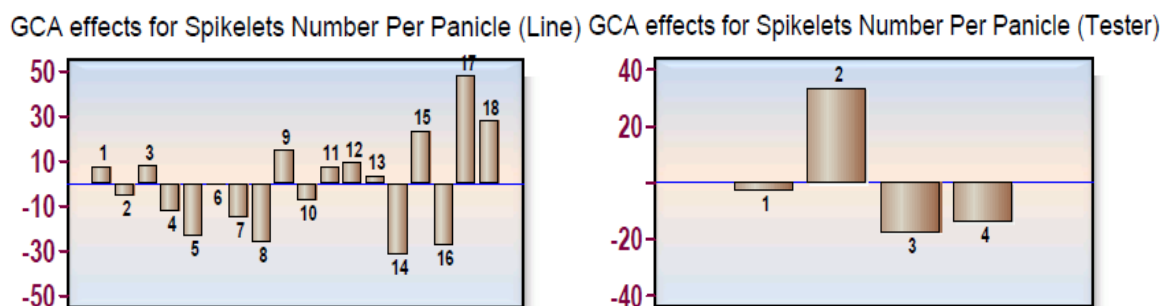
The GCA effects were significant and positive for 5 lines viz. TGMS-14 (1.57), TGMS-17 (1.14), TGMS-15 (1.11), TGMS-12 (0.93) and TGMS-11 (0.92) among these the best combiners for high panicle length was TGMS-14 (1.57). Whereas among the tester the best general combiner was Pant Basmati-1 (1.66) for high panicle length. Similarly **Akram *et al.* (2007)** identified a good general combiner genotype for panicle length to improve rice yield.



SCA effects were positive and significant for eleven crosses out of which the top five crosses were TGMS-1 × UPRI-93-287R (2.11), TGMS-16 × Pant Dhan-4 (1.59), TGMS-4 × Pant Dhan-12 (1.57), TGMS-3 × Pant Basmati-1 (1.38) and TGMS-12 × Pant Basmati-1 (1.30) for high panicle length. **Gnanasekaran *et al.* (2006)** reported a specific combiner of rice which showed desirable SCA effects for panicle length.

4.1.2.5 Spikelet number per panicle

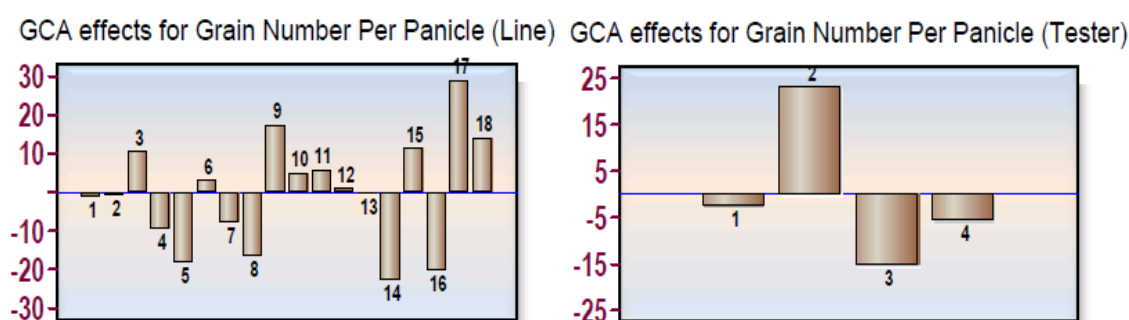
The gca effects were significant and positive for ten lines. The analysis revealed that the lines viz. TGMS-17 (47.83), TGMS-18 (27.68), TGMS-15 (23.28), TGMS-9 (14.63) and TGMS-12 (9.18) were the best general combiners for high spikelet number per panicle. Among the testers the best general combiner was Pant Dhan-12 (33.59).



The sca effects were positive and significant for 23 crosses. Top specific crosses were TGMS-6 × UPRI-93-287R (47.75), TGMS-17 × Pant Basmati-1 (47.75), TGMS-10 × Pant Dhan-4 (42.41), TGMS-16 × Pant Dhan-12 (35.74) and TGMS-16 × UPRI-93-287R (29.30) for more number of spikelet per panicle.

4.1.2.6 Grain number per panicle

The GCA effects were significant and positive for seven lines. The analysis revealed that the lines viz. TGMS-17 (28.81), TGMS-9 (17.81), TGMS-18 (13.91), TGMS-15 (11.31) and TGMS-3 (10.51) were the best general combiners for more grain number per panicle. Whereas among the testers the best general combiner was Pant Dhan-12 (23.19). **Perraju and Sharma (1999)**, **Swamy *et al.* (2003)** and **Gnanasekaran *et al.* (2006)** identified parents with positive GCA effects for number of grains per panicle.

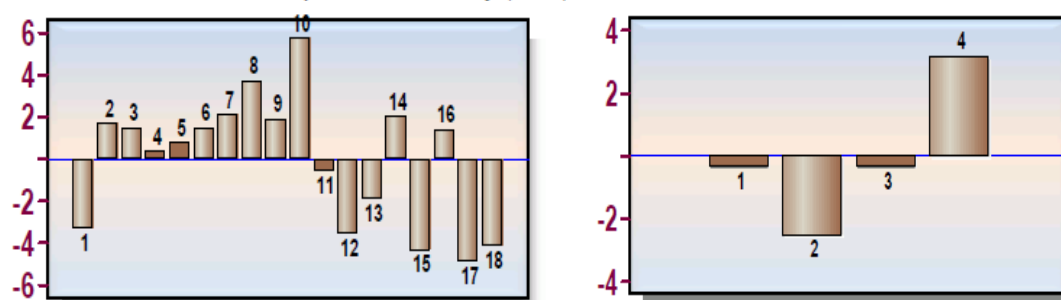


The sca effects were positive and significant for 26 crosses. Top specific crosses were TGMS-10 × Pant Dhan-4 (40.40), TGMS-17 × Pant Basmati-1 (34.35), TGMS-6 × UPRI-93-287R (32.47), TGMS-16 × UPRI-93-287R (32.23) and TGMS-1 × Pant Dhan-12 (27.17) for more number of grains per panicle. **Gnanasekaran *et al.* (2006)** reported better hybrid combinations which showed positive SCA effects for grain number per panicle.

4.1.2.7 Percent spikelet fertility

The GCA effects were significant and positive for eight lines. The analysis revealed that the lines viz. TGMS-10 (5.76), TGMS-8 (3.70), TGMS-7 (2.10), TGMS-14 (2.01) and TGMS-9 (1.89) were the best combiners for high spikeletes fertility or seed setting. Among the testers the best combiner was UPRI-93-287R (3.17). **Swamy *et al.* (2003)** identified two good combiner lines which showed positive gca effect for fertility percentage in rice.

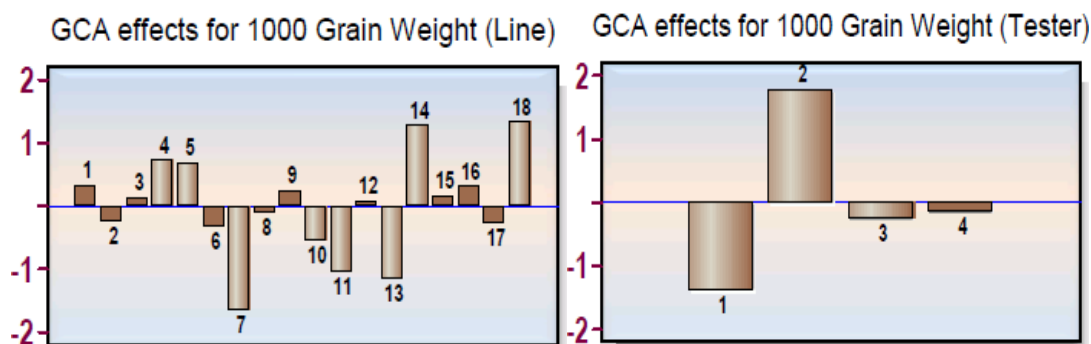
GCA effects for Percent Spikelets Fertility (Line) GCA effects for Percent Spikelets Fertility (Tester)



The SCA effects were positive and significant for 20 crosses. Top specific crosses were TGMS-1 $\times$ UPRI-93-287R (10.67), TGMS-14 $\times$ Pant Dhan-4 (6.96), TGMS-5 $\times$ Pant Basmati-1 (6.61), TGMS-18 $\times$ UPRI-93-287R (6.59) and TGMS-13 $\times$ Pant Dhan-12 (6.47) for high seed setting. The genotypes with high fertility can be developed if recombination breeding coupled with pedigree method of selection is followed. These results are in line with **Panwar (2005)** who adjudged some best hybrids based on high SCA effects and mean performance for spikelet fertility from line $\times$ tester experiment.

4.1.2.8 1000 grain weight

The analysis revealed that the gca effects were significant and positive for three lines viz. TGMS-18 (1.33), TGMS-14 (1.28), TGMS-4 (0.73) and TGMS-5 (0.69) were the best combiners for high grain weight per 1000. **Mehla *et al.* (2000)**, **Rosamma and Vijayakumar (2005)** and **Kumar *et al.* (2007)** identified number of parents having positive GCA effect for 1000-grain weight. Among the testers the best combiner was Pant Dhan-4 (1.78).

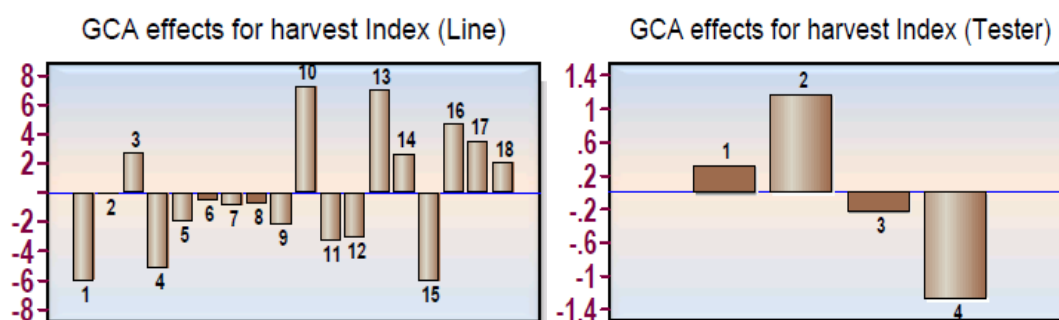


The sca effects were positive and significant for 24 crosses. Top specific crosses were TGMS-4 $\times$ Pant Dhan-4 (3.14), TGMS-15 $\times$ Pant Basmati-1 (2.55), TGMS-15 $\times$ Pant Dhan-4 (2.47), TGMS-5 $\times$ Pant Dhan-12 (2.32) and TGMS-4 $\times$ Pant Dhan-12 (2.30) for

high grain weight. These results are in line with the findings of **Rosamma and Vijayakumar (2005)** and **Sharma (2006)** that identified various good general combiners for the improvement of 1000-grain weight in rice.

4.1.2.9 Harvest index

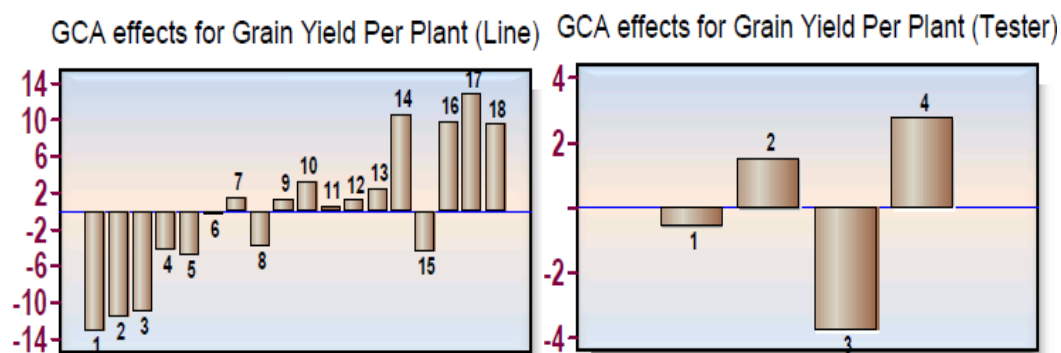
The analysis revealed that the gca effects were significant and positive for eight lines. The top four promising combiners were viz. TGMS-10 (7.27), TGMS-13 (6.97), TGMS-16 (4.70) and TGMS-17 (3.49) for high harvest index. Among the testers the best combiners was Pant Dhan-4 (1.17). Results of **Singh *et al.* (1998)** and **Gnanasekaran *et al.* (2006)**, **Kumar *et al.* (2007)** and **Sanghera and Hussain, (2012)** on number of best combiner lines for harvest index in rice were in favors of our results.



The SCA effects of were positive and significant for 25 crosses. Top specific crosses were TGMS-16 × Pant Basmati-1 (11.18), TGMS-1 × Pant Dhan-12 (9.53), TGMS-18 × Pant Dhan-4 (7.31), TGMS-15 × Pant Dhan-4 (7.26) and TGMS-6 × Pant Dhan-4 (6.68) for high harvest index. Hybrid showing positive SCA effects for harvest index was also reported by **Gnanasekaran *et al.* (2006)**.

4.1.2.10 Grain yield per plant

The analysis revealed that the GCA effects were significant and positive for nine lines of which the top five best combiners were viz. TGMS-17 (12.80), TGMS-14 (10.60), TGMS-16 (9.80), TGMS-18 (9.50) and TGMS-10 (3.30) for high grain yield per plant. Among the testers the best combiners were UPRI-93-287R (2.74) and Pant Dhan-4 (1.52). **Punitha *et al.* (2004)**, **Baskheti, (2005)**, **Manonmani *et al.* (2005)**, **Sharma, (2006)**, **Akram *et al.* (2007)**, **Akter *et al.* (2010)**, **Malik and Singh (2013)** and **Raju *et al.*, (2014)** documented several promising genotypes with significantly high GCA effects in rice.



The SCA effects were positive and significant for 25 crosses. Top five specific crosses were TGMS-9 × Pant Basmati-1 (21.88), TGMS-14 × Pant Dhan-4 (21.28), TGMS-14 × Pant Basmati-1 (16.27), TGMS-16 × UPRI-93-287R (13.24) and TGMS-18 × Pant Dhan-12 (10.44) for higher grain yield per plant. Several crosses having high SCA effects for grain yield per plant in rice has been reported by number of researchers **Mehla *et al.* (2000)**; **Sarker *et al.* (2002)**; **Saxena *et al.* (2002)**; **Manonmani *et al.* (2005)**; **Sharma *et al.* (2005)** and **Petchiammal and Kumar, (2007)**, **Akter *et al.* (2010)**, **Gulzar *et al.* (2012)**, **Thakare *et al.*, (2013)**, **Dorosti and Monajjem (2014)**.

Table 4.4: Ranking of desirable parents on the basis of gca effect for 10 attributes in a Line x Tester fashion

Character	General combiner based on gca effect		Common good general combinations for number of traits	
	Among Lines	Among Testers	Lines	Testers
Days to flowering (50%)	TGMS-16, TGMS-13, TGMS-9 and TGMS-14	Pant Dhan-12 and UPRI-93-287R	TGMS-14, TGMS-9	Pant Dhan-12, UPRI-93-287R
Plant height	TGMS-10, TGMS-14, TGMS-7, TGMS-4 and TGMS-3	Pant Dhan-4, Pant Dhan-12 and UPRI-93-287R	TGMS-14, TGMS-10	Pant Dhan-12, Pant Dhan-4, UPRI-93-287R
Panicle number per plant	TGMS-4, TGMS-5, TGMS-16, TGMS-8 and TGMS-7	Pant Dhan-4 and Pant Dhan-12	TGMS-4	Pant Dhan-12, Pant Dhan-4
Panicle length	TGMS-14, TGMS-17, TGMS-15, TGMS-12 and TGMS-11	Pant Basmati-1, UPRI-93-287R and Pant Dhan-4	TGMS-14, TGMS-17	Pant Dhan-4, UPRI-93-287R
Spikelets number per panicle	TGMS-17, TGMS-18, TGMS-15, TGMS-9 and TGMS-12	Pant Dhan-12	TGMS-17, TGMS-9, TGMS-18	Pant Dhan-12
Grain number per panicle	TGMS-17, TGMS-9, TGMS-18, TGMS-15 and TGMS-3	Pant Dhan-12	TGMS-17, TGMS-9, TGMS-18	Pant Dhan-12
Percent spikeletes fertility	TGMS-10, TGMS-8, TGMS-7, TGMS-14 and TGMS-9	UPRI-93-287R	TGMS-9, TGMS-10	UPRI-93-287R
1000 Grain weight	TGMS-10, TGMS-18, TGMS-14, TGMS-4 and TGMS-5	Pant Dhan-12	TGMS-10, TGMS-4, TGMS-18	Pant Dhan-12
Harvest index	TGMS-10, TGMS-13, TGMS-4, TGMS-16 and TGMS-17	Pant Dhan-4 and Pant Basmati-1	TGMS-17, TGMS-4	Pant Dhan-4
Grain yield per plant	TGMS-17, TGMS-14, TGMS-15, TGMS-18 and TGMS-10	UPRI-93-287R and Pant Dhan-4	TGMS-14, TGMS-10, TGMS-18	Pant Dhan-4, UPRI-93-287R

There were some common lines which were found good general combiner for several traits viz; TGMS-14 was good general combiner for days to 50% flowering, plant height, panicle length and grain yield per plant, TGMS-17 was good combiner for panicle length, spikeletes number per panicle, grain number per panicle and harvest index, TGMS-10 was also good combiner for plant height, percent spikelet fertility, 1000 grain weight and grain yield per plant and another promising line TGMS-18 was good combiner for spikelet number per panicle, grain number per panicle, 1000 grain weight and grain yield per plant (Table 4.4). Among the testers Pant Dhan-12 was the good combiner for days to 50% flowering, plant height, panicle number per plant, spikeletes number per panicle, grain number per panicle and 1000 grain weight, Pant Dhan-4 for plant height, panicle number per plant, panicle length, harvest index and grain yield per plant and UPRI-93-287R was also good combiner for days to 50% flowering, plant height, panicle length and grain yield per plant.

The GCA variance is principally a function of the additive genetic variance and represents a fixable portion of genetic variation. If epistasis is present, GCA will also include additive $\times$ additive type of non-allelic interaction (**Singh and Narayanan, 2004**). The genotypes with significant and desirable GCA effects possess high breeding value therefore the lines with high GCA effects identified in current results are recommended for use in multiple crossing programme to select superior genotypes in segregating generation for the development of high yielding cultivars (**Nadarajan and Gunasekaran, 2005**). Similar suggestions had already been given for the improvement of various yield and yield components (**Boby and Nadarajan, 1993; Vaithiyalingan and Nadarajan, 2005**).

It is inferred that significant GCA and SCA effects for yield components generally result in desirable combining ability effects for yield. However, present results reveals that in some of the crosses, significant SCA effects even for many yield components are not associated with significant SCA effects for yield per plant. Similarly good general combiners among parents do not always exhibit high GCA effects for yield.

Table 4.5: Ranking of desirable cross combinations on the basis of per se performance and sca effect for 10 attributes in a Line x Tester fashion

Character	Crosses <i>per se</i> performance	General combiner based on sca effect	Common good general combinations based on sca effect & <i>Per se</i> performance
Days to flowering (50%)	TGMS-16 × Pant Basmati-1, TGMS-14 × Pant Dhan-12, TGMS-16 × Pant Dhan-4, TGMS-12 × UPRI-93-287R and TGMS-9 × UPRI-93-287R	TGMS-16 × Pant Basmati-1, TGMS-4 × UPRI-93-287R, TGMS-14 × Pant Dhan-12, TGMS-18 × Pant Basmati-1 and TGMS-12 × UPRI-93-287R.	TGMS-16 × Pant Basmati-1, TGMS-14 × Pant Dhan-12 and TGMS-12 × UPRI-93-287R.
Plant height	TGMS-14 × Pant Dhan-12, TGMS-4 × Pant Dhan-4, TGMS-11 × Pant Dhan-12, TGMS-12 × Pant Dhan-4 and TGMS-12 × Pant Dhan-12	TGMS-16 × Pant Basmati-1, TGMS-11 × Pant Dhan-12, TGMS-1 × Pant Basmati-1, TGMS-11 × Pant Dhan-4 and TGMS-4 × Pant Dhan-4.	TGMS-4 × Pant Dhan-4 and TGMS-11 × Pant Dhan-12
Panicle number per plant	TGMS-4 × Pant Dhan-4, TGMS-7 × UPRI-93-287R, TGMS-4 × Pant Dhan-12, TGMS-5 × Pant Dhan-4 and TGMS-14 × Pant Dhan-4	TGMS-4 × Pant Dhan-4, TGMS-7 × UPRI-93-287R, TGMS-18 × Pant Basmati-1, TGMS-14 × Pant Dhan-4 and TGMS-17 × UPRI-93-287R.	TGMS-4 × Pant Dhan-4 and TGMS-7 × UPRI-93-287R
Panicle length	TGMS-12 × Pant Basmati-1, TGMS-14 × Pant Basmati-1, TGMS-11 × Pant Basmati-1 and TGMS-17 × Pant Basmati-1	TGMS-1 × UPRI-93-287R, TGMS-16 × Pant Dhan-4, TGMS-4 × Pant Dhan-12, TGMS-3 × Pant Basmati-1 and TGMS-12 × Pant Basmati-1.	TGMS-12 × Pant Basmati-1
Spikelets number per panicle	TGMS-17 × Pant Basmati-1, TGMS-17 × Pant Dhan-4, TGMS-18 × Pant Dhan-4, TGMS-10 × Pant Dhan-4 and TGMS-1 × Pant Dhan-4	TGMS-6 × UPRI-93-287R, TGMS-17 × Pant Basmati-1, TGMS-10 × Pant Dhan-4, TGMS-16 × Pant Dhan-12 and TGMS-16 × UPRI-93-287R.	TGMS-17 × Pant Basmati-1 and TGMS-10 × Pant Dhan-4
Grain number per panicle	TGMS-10 × Pant Dhan-4, TGMS-17 × Pant Basmati-1, TGMS-11 × Pant Dhan-4, TGMS-9 × Pant Dhan-4 and TGMS-13 × Pant Dhan-4	TGMS-10 × Pant Dhan-4, TGMS-17 × Pant Basmati-1, TGMS-6 × UPRI-93-287R, TGMS-16 × UPRI-93-287R and TGMS-1 × Pant Dhan-12.	TGMS-10 × Pant Dhan-4 and TGMS-17 × Pant Basmati-1
Percent spikeletes fertility	TGMS-1 × UPRI-93-287R, TGMS-10 × UPRI-93-287R, TGMS-2 × UPRI-93-287R, TGMS-16 × UPRI-93-287R and TGMS-8 × UPRI-93-287R	TGMS-1 × UPRI-93-287R, TGMS-14 × Pant Dhan-4, TGMS-5 × Pant Basmati-1, TGMS-18 × UPRI-93-287R and TGMS-13 × Pant Dhan-12.	TGMS-1 × UPRI-93-287R
1000 grain weight	TGMS-4 × Pant Dhan-4, TGMS-18 × Pant Dhan-4, TGMS-15 × Pant Dhan-4, TGMS-3 × Pant Dhan-4 and TGMS-14 × UPRI-93-287R	TGMS-4 × Pant Dhan-4, TGMS-15 × Pant Basmati-1, TGMS-15 × Pant Dhan-4, TGMS-5 × Pant Dhan-12 and TGMS-4 × Pant Dhan-12	TGMS-4 × Pant Dhan-4 and TGMS-15 × Pant Dhan-4
Harvest index	TGMS-16 × Pant Basmati-1, TGMS-13 × Pant Basmati-1, TGMS-10 × Pant Dhan-4, TGMS-18 × Pant Dhan-4 and TGMS-10 × Pant Dhan-12	TGMS-16 × Pant Basmati-1, TGMS-1 × Pant Dhan-12, TGMS-18 × Pant Dhan-4, TGMS-15 × Pant Dhan-4 and TGMS-6 × Pant Dhan-4	TGMS-16 × Pant Basmati-1 and TGMS-18 × Pant Dhan-4
Grain yield per plant	TGMS-14 × Pant Dhan-4, TGMS-16 × UPRI-93-287R, TGMS-17 × UPRI-93-287R, TGMS-9 × Pant Basmati-1 and TGMS-14 × UPRI-93-287R	TGMS-9 × Pant Basmati-1, TGMS-14 × Pant Dhan-4, TGMS-14 × Pant Basmati-1, TGMS-16 × UPRI-93-287R and TGMS-18 × Pant Dhan-12	TGMS-14 × Pant Dhan-4, TGMS-9 × Pant Basmati-1 and TGMS-16 × UPRI-93-287R

Based on the SCA effect and *per se* performance the common crosses were selected for traits (Table-4.5). For days to 50% flowering TGMS-16 × Pant Basmati-1, TGMS-14 × Pant Dhan-12 and TGMS-12 × UPRI-93-287R were the best hybrids for early maturity, TGMS-4 × Pant Dhan-4 and TGMS-11 × Pant Dhan-12 for dwarf plant type, for panicle number per plant TGMS-4 × Pant Dhan-4 and TGMS-7 × UPRI-93-287R, for panicle length was TGMS-12 × Pant Basmati-1, good hybrids for spikeletes number per panicle and grain number per panicle were TGMS-17 × Pant Basmati-1 and TGMS-10 × Pant Dhan-4, for percent spikeletes fertility was TGMS-1 × UPRI-93-287R, for 1000 grain weight were TGMS-4 × Pant Dhan-4 and TGMS-15 × Pant Dhan-4, for harvest index TGMS-16 × Pant Basmati-1 and TGMS-18 × Pant Dhan-4 and for grain yield per plant good hybrids were TGMS-14 × Pant Dhan-4, TGMS-9 × Pant Basmati-1 and TGMS-16 × UPRI-93-287R. **Chandirakala *et al.*, (2008), Saleem *et al.* (2010), Selvaraj *et al.* (2011), Sanghera and Hussain (2012), Malik and Singh (2013) and Raju *et al.*, (2014)** reported several promising specific combiners based on high *per se* performance and SCA effects for grain yield per plant.

Study of crosses in relation to the GCA effects of the parents indicated that for days to 50% flowering. The hybrids TGMS-16 × Pant Basmati-1 (High × High), TGMS-14 × Pant Dhan-12 (High × High) and TGMS-12 × UPRI-93-287R (High × Average) had emerged from high × high and High × Average GCA of parents, whereas in other crosses TGMS-4 × Pant Dhan-4 (High × High) and TGMS-11 × Pant Dhan-12 (Low × High) for dwarf plant type, for panicle number per plant TGMS-4 × Pant Dhan-4 (H × A) and TGMS-7 × UPRI-93-287R (A × A), for panicle length was TGMS-12 × Pant Basmati-1 (L × L), for spikeletes number per panicle and grain number per panicle had TGMS-17 × Pant Basmati-1 (H × H) and TGMS-10 × Pant Dhan-4 (L × L), for per-cent spikeletes fertility was TGMS-1 × UPRI-93-287R (H × L), for 1000 grain weight had TGMS-4 × Pant Dhan-4 (L × H) and TGMS-15 × Pant Dhan-4 (L × L), for harvest index TGMS-16 × Pant Basmati-1 (L × L) and TGMS-18 × Pant Dhan-4 (H × H) and for grain yield per plant good hybrids were TGMS-14 × Pant Dhan-4 (H × H), TGMS-9 × Pant Basmati-1 (H × L) and TGMS-16 × UPRI-93-287R (H × H). Several good SCA crosses for grain yield have been reported by **Tiwari *et al.*, (2011), Divyapriya and Kalaiyarasi (2014)** in different TGMS lines.

Hariprasanna *et al.* (2006) has given genetical causes of manifestation of SCA by hybrids. According to them the expression of high, low or non-significant SCA effects by any type of combination among the parents may be due to differential expression of contributing traits in specific residual genetic back ground. Since yield associated traits are linked either

positively or negatively, therefore it is likely to find GCA in the desired direction for some traits and in undesirable direction for the others. So the value of a parent with respect to GCA for a number of characters has tremendous importance. The pre-eminence of high $\times$ low crosses can be attributed to the fact that such crosses may result in strong transgressive segregants for the desired traits, because of segregation of genes with strong potentials (Iqbal and Khan, 2003). Genes controlling high GCA effects express almost same phenotypes when they are in homozygous state irrespective to residual genetic background in contrast to genes showing low GCA effects which show their potential only in homozygous residual genetic background. Thus high $\times$ high or low $\times$ low combinations generally emerge out from the situations essentially similar to their parents were as high $\times$ low crosses generate heterozygous genotypes, which show high effects and therefore are better to each of the parents. Keeping this in view in the present results it looks that appropriate breeding approach necessitates to start with high $\times$ low and high $\times$ high crosses.

4.1.3 Contribution of lines, testers and line $\times$ tester to the total variance

Contribution of lines, testers and line $\times$ tester interaction is presented in Table 4.6. The contribution of lines to the total variance was greater than testers and line $\times$ tester interaction for three parameters. These were days to flowering (49.28%), spikelets number per panicle (35.51%) and grain yield per plant (51.07%) thus indicating predominant maternal influence for these traits. Contribution of testers was more than lines and line $\times$ tester interactions for two characters viz. plant height (46.15%) and panicle length (42.43%) which has been reported by Rashid *et al.*, (2007). Line $\times$ tester interactions contributed more than lines and testers for five characters viz. panicle number per plant (57.86%), grain number per panicle (42.25%), percent spikelets fertility (58.11%), 1000 grain weight (52.01) and harvest index (55.70). These results are in agreement with the findings of Rashid *et al.* (2007).

Table 4.6: Proportional contribution (%) of lines, testers and their interaction to the total variance

Source	Days to 50% flowering	Plant height	Panicle number per plant	Panicle length	Spikelet number per panicle	Grain number per panicle	Percent spikelet fertility	1000 grain weight	Harvest index	Grain yield per plant
Line	49.28	17.16	38.41	30.12	35.51	27.40	28.33	14.82	42.20	51.07
Tester	4.85	46.15	3.72	42.43	34.66	30.34	13.56	33.16	2.10	5.65
Line $\times$ Tester	45.88	36.69	57.86	27.45	29.82	42.25	58.11	52.01	55.70	43.28

4.2 Determining the nature and magnitude of heterosis in the hybrids

Significant standard heterosis manifested as the percent increase or decrease over the standard variety was present in Table 4.7 to 4.11 for ten characters viz. days to 50% flowering, plant height, panicle length, panicle number per plant, spikelet number per panicle, grain number per panicle, percent spikelet fertility, 1000 grain weight, harvest index and grain yield per plant.

Heterosis is the process by which the performance of an F_1 generated by crossing two genetically different individuals is superior to that of the mean of the parents. Genetically, heterosis is considered mainly to be the result of adding dominant or partially dominant alleles in repulsion phase at loci (**Hallauer and Miranda, 1988**). As such one parent contributes useful alleles at some of the loci, while other parent compliments by providing desirable alleles at the remaining loci. A second type of heterosis can also be visualized, that is crossing two plants with different morphologies. If the loci controlling the morphologies are partially to completely dominant and each affects yield positively, then the morphologically different contribution of each parent in the hybrid may create higher yield progeny by combining morphologies. **Shull (1914)** defined heterosis as expression of increased vigour, size fruitfulness, speed of development, resistance to diseases and to insects, or to climatic rigors kind of manifested by cross bred organism as compared with corresponding inbreds which in the results of unlikelines in the constitution of uniting parental gametes.

Due to poor seed setting in the TGMS lines, the other types of heterosis could not be estimated for all the traits. For estimation of standard heterosis the checks used Pant Sankar Dhan-1, Pant Sankar Dhan-3, UPRH-128 and UPRH-131. Result on the nature and magnitude of standard heterosis for each of the 10 traits individually over the best check among the four are presented below:

4.2.1 Days to 50% flowering

The estimates of per cent standard heterosis over 4 checks of 72 hybrids for days to flowering are presented in the Table 4.7.

Out of the four checks used for estimation the standard heterosis the best check for earliness or early flowering was Pant Sankar Dhan-1. A total of 47 hybrids manifested significant heterosis, out of which 35 were negative estimates. The estimates ranged

between -20.71 (TGMS-16 × Pant Basmati-1) to 16.50 (TGMS-4 × Pant Dhan-12). The hybrids showing high heterosis for earliness were TGMS-16 × Pant Basmati-1 (-20.71), TGMS-14 × Pant Dhan-12 (-15.86), TGMS-16 × Pant Dhan-4 (-13.92), TGMS-12 × UPRI-93-287R (-12.94) and TGMS-9 × UPRI-93-287R (-20.71). Negative heterosis for early maturing was also reported by **Alam *et al.* (2004)**, **Nuruzzaman *et al.* (2002)** and **Rahimi *et al.*, (2010)**.

4.2.2 Plant height

In case of plant height, the standard heterosis has estimated on four checks as like days to flowering. The best check for dwarf plant type was UPRH-131, against which all the cross combinations showed the significant and negative heterosis for dwarf plant type. The estimates of heterosis ranged between the -26.59 (TGMS-14 × Pant Dhan-12) to -3.22 (TGMS-15 × Pant Basmati-1). The five best hybrids showing high heterosis for dwarf plant type were TGMS-14 × Pant Dhan-12 (-26.59), TGMS-4 × Pant Dhan-4 (-26.40), TGMS-11 × Pant Dhan-12 (-25.72), TGMS-12 × Pant Dhan-4 (-25.06) and TGMS-12 × Pant Dhan-12 (-25.02). Negative heterosis for plant height had also been reported by **Patil *et al.* (2003)** and **Chandirakala and Thiyagarajan (2010)**.

Table 4.7: Percent standard heterosis for days to flowering and plant height

Cross	Days to 50% flowering				Plant Height			
	Pant Sankar Dhan-1	Pant Sankar Dhan-3	UPRH-128	UPRH-131	Pant Sankar Dhan-1	Pant Sankar Dhan-3	UPRH-128	UPRH-131
TGMS-1*Pant Basmati-1	18.99**	-0.65	12.04**	1.66	13.90**	-3.92	0.34	-17.62**
TGMS-1*Pant Dhan-4	23.64**	3.24*	16.42**	5.63**	13.07**	-4.62*	-0.39	-18.22**
TGMS-1*Pant Dhan-12	15.89**	-3.24*	9.12**	-0.99	13.01**	-4.67*	-0.45	-18.26**
TGMS-1*UPRI-93-287R	32.56**	10.68**	24.82**	13.25**	13.98**	-3.85	0.41	-17.56**
TGMS-2*Pant Basmati-1	17.83**	-1.62	10.95**	0.66	14.94**	-3.04	1.25	-16.87**
TGMS-2*Pant Dhan-4	12.02**	-6.47**	5.47**	-4.30**	12.59**	-5.03*	-0.82	-18.57**
TGMS-2*Pant Dhan-12	6.59**	-11.00**	0.36	-8.94**	11.49**	-5.95**	-1.79	-19.36**
TGMS-2*UPRI-93-287R	15.50**	-3.56*	8.76**	-1.32	9.57**	-7.57**	-3.48	-20.75**
TGMS-3*Pant Basmati-1	17.83**	-1.62	10.95**	0.66	14.88**	-3.09	1.20	-16.91**
TGMS-3*Pant Dhan-4	9.30**	-8.74**	2.92	-6.62**	8.41**	-8.55**	-4.50*	-21.59**
TGMS-3*Pant Dhan-12	14.73**	-4.21**	8.03**	-1.99	12.53**	-5.08*	-0.87	-18.61**
TGMS-3*UPRI-93-287R	19.77**	0.00	12.77**	2.32	6.59**	-10.09**	-6.11**	-22.91**
TGMS-4*Pant Basmati-1	36.43**	13.92**	28.47**	16.56**	16.50**	-1.73	2.62	-15.74**
TGMS-4*Pant Dhan-4	39.53**	16.50**	31.39**	19.21**	1.77	-14.15**	-10.35**	-26.40**
TGMS-4*Pant Dhan-12	20.54**	0.65	13.50**	2.98	8.78**	-8.24**	-4.18*	-21.33**
TGMS-4*UPRI-93-287R	17.05**	-2.27	10.22**	0.00	12.53**	-5.08*	-0.87	-18.61**
TGMS-5*Pant Basmati-1	22.48**	2.27	15.33**	4.64**	17.19**	-1.14	3.23	-15.24**
TGMS-5*Pant Dhan-4	30.23**	8.74**	22.63**	11.26**	13.48**	-4.27*	-0.03	-17.92**
TGMS-5*Pant Dhan-12	19.77**	0.00	12.77**	2.32	14.17**	-3.69	0.57	-17.43**
TGMS-5*UPRI-93-287R	22.87**	2.59	15.69**	4.97**	13.66**	-4.12*	0.12	-17.79**

TGMS-6*Pant Basmati-1	17.83**	-1.62	10.95**	0.66	15.65**	-2.44	1.87	-16.36**
TGMS-6*Pant Dhan-4	20.16**	0.32	13.14**	2.65	9.63**	-7.52**	-3.43	-20.71**
TGMS-6*Pant Dhan-12	15.12**	-3.88*	8.39**	-1.66	8.78**	-8.24**	-4.18*	-21.33**
TGMS-6*UPRI-93-287R	27.91**	6.80**	20.44**	9.27**	10.28**	-6.97**	-2.85	-20.24**
TGMS-7*Pant Basmati-1	26.36**	5.50**	18.98**	7.95**	17.69**	-0.72	3.68	-14.88**
TGMS-7*Pant Dhan-4	14.34**	-4.53**	7.66**	-2.32	4.81*	-11.59**	-7.67**	-24.20**
TGMS-7*Pant Dhan-12	19.38**	-0.32	12.41**	1.99	6.04*	-10.55**	-6.59**	-23.31**
TGMS-7*UPRI-93-287R	16.28**	-2.91	9.49**	-0.66	8.48**	-8.49**	-4.44*	-21.54**
TGMS-8*Pant Basmati-1	13.95**	-4.85**	7.30**	-2.65	19.78**	1.05	5.52**	-13.36**
TGMS-8*Pant Dhan-4	14.34**	-4.53**	7.66**	-2.32	9.65**	-7.51**	-3.41	-20.70**
TGMS-8*Pant Dhan-12	25.97**	5.18**	18.61**	7.62**	9.32**	-7.78**	-3.70	-20.93**
TGMS-8*UPRI-93-287R	7.75**	-10.03**	1.46	-7.95**	15.77**	-2.34	1.98	-16.27**
TGMS-9*Pant Basmati-1	18.60**	-0.97	11.68**	1.32	24.00**	4.60*	9.23**	-10.32**
TGMS-9*Pant Dhan-4	13.95**	-4.85**	7.30**	-2.65	5.59*	-10.92**	-6.98**	-23.63**
TGMS-9*Pant Dhan-12	12.02**	-6.47**	5.47**	-4.30**	14.23**	-3.64	0.62	-17.38**
TGMS-9*UPRI-93-287R	4.65*	-12.62**	-1.46	-10.60**	8.01**	-8.89**	-4.86*	-21.88**
TGMS-10*Pant Basmati-1	17.05**	-2.27	10.22**	0.00	16.17**	-2.00	2.34	-15.98**
TGMS-10*Pant Dhan-4	15.89**	-3.24*	9.12**	-0.99	8.67**	-8.33**	-4.27*	-21.40**
TGMS-10*Pant Dhan-12	14.73**	-4.21**	8.03**	-1.99	4.69	-11.69**	-7.78**	-24.28**
TGMS-10*UPRI-93-287R	11.63**	-6.80**	5.11**	-4.64**	4.93*	-11.49**	-7.57**	-24.11**
TGMS-11*Pant Basmati-1	24.03**	3.56*	16.79**	5.96**	31.98**	11.34**	16.26**	-4.54**
TGMS-11*Pant Dhan-4	33.72**	11.65**	25.91**	14.24**	4.34	-11.98**	-8.09**	-24.54**
TGMS-11*Pant Dhan-12	15.12**	-3.88*	8.39**	-1.66	2.70	-13.37**	-9.53**	-25.72**
TGMS-11*UPRI-93-287R	16.67**	-2.59	9.85**	-0.33	15.34**	-2.70	1.61	-16.58**
TGMS-12*Pant Basmati-1	18.60**	-0.97	11.68**	1.32	23.82**	4.45*	9.07**	-10.45**
TGMS-12*Pant Dhan-4	18.60**	-0.97	11.68**	1.32	3.61	-12.60**	-8.73**	-25.06**
TGMS-12*Pant Dhan-12	15.50**	-3.56*	8.76**	-1.32	3.67	-12.55**	-8.68**	-25.02**
TGMS-12*UPRI-93-287R	4.26*	-12.94**	-1.82	-10.93**	12.54**	-5.06*	-0.86	-18.60**
TGMS-13*Pant Basmati-1	5.04**	-12.30**	-1.09	-10.26**	17.17**	-1.16	3.21	-15.26**
TGMS-13*Pant Dhan-4	6.98**	-10.68**	0.73	-8.61**	10.54**	-6.75**	-2.62	-20.05**
TGMS-13*Pant Dhan-12	9.30**	-8.74**	2.92	-6.62**	5.75*	-10.79**	-6.84**	-23.51**
TGMS-13*UPRI-93-287R	8.53**	-9.39**	2.19	-7.28**	12.18**	-5.37**	-1.18	-18.86**
TGMS-14*Pant Basmati-1	21.71**	1.62	14.60**	3.97*	20.75**	1.86	6.37**	-12.66**
TGMS-14*Pant Dhan-4	16.28**	-2.91	9.49**	-0.66	7.52**	-9.30**	-5.28*	-22.23**
TGMS-14*Pant Dhan-12	0.78	-15.86**	-5.11**	-13.91**	1.50	-14.38**	-10.59**	-26.59**
TGMS-14*UPRI-93-287R	12.40**	-6.15**	5.84**	-3.97*	4.86*	-11.54**	-7.62**	-24.16**
TGMS-15*Pant Basmati-1	25.58**	4.85**	18.25**	7.28**	33.81**	12.87**	17.87**	-3.22
TGMS-15*Pant Dhan-4	20.54**	0.65	13.50**	2.98	18.08**	-0.39	4.02	-14.60**
TGMS-15*Pant Dhan-12	20.54**	0.65	13.50**	2.98	11.45**	-5.98**	-1.82	-19.39
TGMS-15*UPRI-93-287R	13.95**	-4.85**	7.30**	-2.65	13.64**	-4.14*	0.11	-17.81
TGMS-16*Pant Basmati-1	-5.04**	-20.71**	-10.58**	-18.87**	10.30**	-6.96**	-2.84	-20.23**
TGMS-16*Pant Dhan-4	3.10	-13.92**	-2.92	-11.92**	17.58**	-0.82	3.57	-14.96**
TGMS-16*Pant Dhan-12	8.91**	-9.06**	2.55	-6.95**	11.76**	-5.73**	-1.55	-19.17**
TGMS-16*UPRI-93-287R	15.89**	-3.24*	9.12**	-0.99	15.87**	-2.25	2.07	-16.19**
TGMS-17*Pant Basmati-1	18.99**	-0.65	12.04**	1.66	23.09**	3.83	8.43**	-10.97**
TGMS-17*Pant Dhan-4	17.44**	-1.94	10.58**	0.33	10.13**	-7.10**	-2.98	-20.34**
TGMS-17*Pant Dhan-12	3.10	-13.92**	-2.92	-11.92**	10.50**	-6.79**	-2.66	-20.08**
TGMS-17*UPRI-93-287R	12.79**	-5.83**	6.20**	-3.64*	14.41**	-3.49	0.79	-17.25**
TGMS-18*Pant Basmati-1	6.59**	-11.00**	0.36	-8.94**	19.80**	1.06	5.54**	-13.35**
TGMS-18*Pant Dhan-4	19.77**	0.00	12.77**	2.32	16.13**	-2.03	2.30	-16.00**
TGMS-18*Pant Dhan-12	15.89**	-3.24*	9.12**	-0.99	10.30**	-6.96**	-2.84	-20.23**
TGMS-18*UPRI-93-287R	15.50**	-3.56*	8.76**	-1.32	10.50**	-6.79**	-2.66	-20.08**

4.2.3 Panicle number per plant

Out of the four checks used for estimation the standard heterosis. The best check for more panicle number per plant was Pant Sankar Dhan-1. 70 hybrid combinations manifested significant and positive heterosis (Table- 4.8). The estimates ranged between 2.42 (TGMS-2 × Pant Dhan-4) to 154.76 (TGMS-4 × Pant Dhan-4). Some of the best hybrids showing high heterosis for more panicles were TGMS-4 × Pant Dhan-4 (154.76), TGMS-7 × UPRI-93-287R (107.14), TGMS-4 × Pant Dhan-12 (104.72), TGMS-5 × Pant Dhan-4 (90.44) and TGMS-14 × Pant Dhan-4 (83.33). High heterosis for more number of panicles per plant has been reported by **Patil *et al.* (2003)** and **Chandirakala and Thiyagarajan (2010)**.

4.2.4 Panicle length

The best check for high panicle length was Pant Sankar Dhan-1 against which 70 cross combinations showed the significant and positive heterosis for Panicle length. The estimates of heterosis ranged between the -2.91 (TGMS-8 × UPRI-93-287R) to 30.86 (TGMS-12 × Pant Basmati-1). The five best hybrids showing high heterosis for panicle length were TGMS-12 × Pant Basmati-1 (30.86), TGMS-14 × Pant Basmati-1 (28.27), TGMS-11 × Pant Basmati-1 (26.99), TGMS-17 × Pant Basmati-1 (25.93) and TGMS-12 × Pant Basmati-1 (25.77). Significant standard heterosis for panicle length had also been reported by **Banumathy *et al.* (2003)** and **Chandirakala and Thiyagarajan (2010)**.

Table 4.8: Percent standard heterosis for panicle number per plant and panicle length

Cross	Panicle number per plant				Panicle length			
	Pant Sankar Dhan-1	Pant Sankar Dhan-3	UPRH-128	UPRH-131	Pant Sankar Dhan-1	Pant Sankar Dhan-3	UPRH-128	UPRH-131
TGMS-1*Pant Basmati-1	45.24**	-1.61	-4.69*	38.64**	7.59**	-9.39**	2.62	-21.55**
TGMS-1*Pant Dhan-4	80.95**	22.58**	18.75**	72.73**	10.49**	-6.95**	5.38*	-19.44**
TGMS-1*Pant Dhan-12	33.29**	-9.70**	-12.53**	27.23**	5.51*	-11.15**	0.63	-23.08**
TGMS-1*UPRI-93-287R	33.29**	-9.70**	-12.53**	27.23**	13.89**	-4.08*	8.63**	-16.96**
TGMS-2*Pant Basmati-1	19.05**	-19.35**	-21.88**	13.64**	17.12**	-1.36	11.71**	-14.61**
TGMS-2*Pant Dhan-4	2.42	-30.62**	-32.79**	-2.23	16.32**	-2.04	10.94**	-15.19**
TGMS-2*Pant Dhan-12	19.09**	-19.33**	-21.85**	13.67**	9.94**	-7.41**	4.85*	-19.85**
TGMS-2*UPRI-93-287R	16.63**	-20.99**	-23.46**	11.33**	8.08**	-8.98**	3.08	-21.20**
TGMS-3*Pant Basmati-1	9.52**	-25.81**	-28.13**	4.55	23.59**	4.08*	17.87**	-9.89**
TGMS-3*Pant Dhan-4	42.86**	-3.23	-6.25**	36.36**	13.18**	-4.68*	7.95**	-17.48**
TGMS-3*Pant Dhan-12	64.29**	11.29**	7.81**	56.82**	5.33*	-11.29**	0.46	-23.20**
TGMS-3*UPRI-93-287R	14.29**	-22.58**	-25.00**	9.09**	3.22	-13.07**	-1.55	-24.74**
TGMS-4*Pant Basmati-1	61.90**	9.68**	6.25**	54.55**	19.31**	0.48	13.79**	-13.02**

TGMS-4*Pant Dhan-4	154.76**	72.58**	67.19**	143.18**	13.17**	-4.69*	7.94**	-17.49**
TGMS-4*Pant Dhan-12	104.72**	38.68**	34.35**	95.42**	18.34**	-0.34	12.87**	-13.72**
TGMS-4*UPRI-93-287R	49.96**	1.59	-1.59	43.14**	8.64**	-8.50**	3.62	-20.79**
TGMS-5*Pant Basmati-1	42.86**	-3.23	-6.25**	36.36**	21.31**	2.17	15.70**	-11.55**
TGMS-5*Pant Dhan-4	90.44**	29.01**	24.97**	81.78**	15.59**	-2.65	10.25**	-15.72**
TGMS-5*Pant Dhan-12	66.63**	12.88**	9.35**	59.05**	16.01**	-2.30	10.64**	-15.42**
TGMS-5*UPRI-93-287R	50.00**	1.61	-1.56	43.18**	14.05**	-3.95	8.78**	-16.84**
TGMS-6*Pant Basmati-1	16.63**	-20.99**	-23.46**	11.33**	22.46**	3.13	16.80**	-10.72**
TGMS-6*Pant Dhan-4	19.05**	-19.35**	-21.88**	13.64**	15.43**	-2.79	10.09**	-15.84**
TGMS-6*Pant Dhan-12	69.05**	14.52**	10.94**	61.36**	5.83*	-10.87**	0.94	-22.84**
TGMS-6*UPRI-93-287R	38.06**	-6.48**	-9.40**	31.78**	7.01**	-9.88**	2.07	-21.98**
TGMS-7*Pant Basmati-1	28.57**	-12.90**	-15.63**	22.73**	24.88**	5.17*	19.11**	-8.95**
TGMS-7*Pant Dhan-4	23.81**	-16.13**	-18.75**	18.18**	16.46**	-1.92	11.08**	-15.09**
TGMS-7*Pant Dhan-12	35.67**	-8.09**	-10.96**	29.51**	8.95**	-8.24**	3.92	-20.56**
TGMS-7*UPRI-93-287R	107.14**	40.32**	35.94**	97.73**	8.64**	-8.50**	3.62	-20.79**
TGMS-8*Pant Basmati-1	42.82**	-3.25	-6.28**	36.33**	17.61	-0.95	12.17**	-14.25**
TGMS-8*Pant Dhan-4	76.19**	19.35**	15.63**	68.18**	6.77**	-10.08**	1.84	-22.15**
TGMS-8*Pant Dhan-12	26.19**	-14.52**	-17.19**	20.45**	7.04**	-9.85**	2.09	-21.96**
TGMS-8*UPRI-93-287R	61.87**	9.65**	6.22**	54.51**	2.91	-13.33**	-1.85	-24.97**
TGMS-9*Pant Basmati-1	42.90**	-3.20	-6.22**	36.40**	24.97**	5.25*	19.20**	-8.88**
TGMS-9*Pant Dhan-4	33.33**	-9.68**	-12.50**	27.27**	7.27**	-9.66**	2.31	-21.79**
TGMS-9*Pant Dhan-12	30.95**	-11.29**	-14.06**	25.00**	15.41**	-2.80	10.08**	-15.85**
TGMS-9*UPRI-93-287R	16.67**	-20.97**	-23.44**	11.36**	9.85**	-7.48**	4.78*	-19.91**
TGMS-10*Pant Basmati-1	26.19**	-14.52**	-17.19**	20.45**	21.32**	2.18	15.72**	-11.54**
TGMS-10*Pant Dhan-4	40.44**	-4.87*	-7.84**	34.05**	20.69**	1.64	15.11**	-12.00**
TGMS-10*Pant Dhan-12	57.18**	6.48**	3.15	50.04**	11.32**	-6.25**	6.18**	-18.84**
TGMS-10*UPRI-93-287R	40.48**	-4.84*	-7.81**	34.09**	10.57**	-6.88**	5.46*	-19.39**
TGMS-11*Pant Basmati-1	28.57**	-12.90**	-15.63**	22.73**	26.99**	6.95**	21.12**	-7.41**
TGMS-11*Pant Dhan-4	28.57**	-12.90**	-15.63**	22.73**	14.30**	-3.74	9.01**	-16.67**
TGMS-11*Pant Dhan-12	40.44**	-4.87*	-7.84**	34.05**	16.64**	-1.77	11.25**	-14.96**
TGMS-11*UPRI-93-287R	66.67**	12.90**	9.38**	59.09**	17.61**	-0.95	12.17**	-14.25**
TGMS-12*Pant Basmati-1	30.99**	-11.26**	-14.04**	25.04**	30.86**	10.20**	24.81**	-4.59**
TGMS-12*Pant Dhan-4	42.86**	-3.23	-6.25**	36.36**	14.46**	-3.61	9.17**	-16.55**
TGMS-12*Pant Dhan-12	38.10**	-6.45**	-9.38**	31.82**	13.33**	-4.56*	8.09**	-17.37**
TGMS-12*UPRI-93-287R	42.86**	-3.23	-6.25**	36.36**	17.04**	-1.43	11.63**	-14.66**
TGMS-13*Pant Basmati-1	30.95**	-11.29**	-14.06**	25.00	20.19**	1.22	14.64**	-12.37**
TGMS-13*Pant Dhan-4	9.48**	-25.83**	-28.15**	4.51	16.40**	-1.97	11.02**	-15.14**
TGMS-13*Pant Dhan-12	52.34**	3.20	-0.03	45.42**	8.56**	-8.57**	3.54	-20.85**
TGMS-13*UPRI-93-287R	45.24**	-1.61	-4.69*	38.64**	13.73**	-4.22*	8.47**	-17.08**
TGMS-14*Pant Basmati-1	14.29**	-22.58**	-25.00**	9.09**	28.27**	8.03**	22.34**	-6.48**
TGMS-14*Pant Dhan-4	83.33**	24.19**	20.31**	75.00**	19.16**	0.35	13.65**	-13.12**
TGMS-14*Pant Dhan-12	30.95**	-11.29**	-14.06**	25.00**	18.00**	-0.62	12.54**	-13.97**
TGMS-14*UPRI-93-287R	54.76**	4.84*	1.56	47.73**	20.58**	1.55	15.01**	-12.08**
TGMS-15*Pant Basmati-1	69.01**	14.49**	10.91**	61.33**	25.77**	5.92**	19.95**	-8.30**
TGMS-15*Pant Dhan-4	35.71**	-8.06**	-10.94**	29.55**	25.28**	5.51**	19.49**	-8.66**
TGMS-15*Pant Dhan-12	45.24**	-1.61	-4.69*	38.64**	18.35**	-0.33	12.88**	-13.71**
TGMS-15*UPRI-93-287R	35.67**	-8.09**	-10.96**	29.51**	9.21**	-8.03**	4.16	-20.38**
TGMS-16*Pant Basmati-1	50.00**	1.61	-1.56	43.18**	14.78**	-3.33	9.48**	-16.31**
TGMS-16*Pant Dhan-4	64.29**	11.29**	7.81**	56.82**	11.87**	-5.78**	6.70**	-18.43**
TGMS-16*Pant Dhan-12	54.76**	4.84*	1.56	47.73**	17.06**	-1.42	11.65**	-14.65**
TGMS-16*UPRI-93-287R	61.94**	9.70**	6.28**	54.58**	10.43**	-7.00**	5.33*	-19.48**
TGMS-17*Pant Basmati-1	14.29**	-22.58**	-25.00**	9.09**	25.93**	6.05**	20.11**	-8.19**
TGMS-17*Pant Dhan-4	2.42	-30.62**	-32.79**	-2.23	19.95**	1.02	14.41**	-12.54**

TGMS-17*Pant Dhan-12	50.00**	1.61	-1.56	43.18**	18.16**	-0.49	12.70**	-13.85**
TGMS-17*UPRI-93-287R	66.71**	12.93**	9.40**	59.13**	15.02**	-3.13	9.71**	-16.14**
TGMS-18*Pant Basmati-1	59.52**	8.06**	4.69*	52.27**	20.18**	1.21	14.63**	-12.38**
TGMS-18*Pant Dhan-4	19.05**	-19.35**	-21.88**	13.64**	19.06**	0.27	13.56**	-13.19**
TGMS-18*Pant Dhan-12	28.57**	-12.90**	-15.63**	22.73**	7.03**	-9.86**	2.08	-21.97**
TGMS-18*UPRI-93-287R	21.43**	-17.74**	-20.31**	15.91**	10.49**	-6.95**	5.38*	-19.44**

4.2.5 Spikelet number per panicle

The best check for more spikelet number per panicle was Pant Sankar Dhan-3. Out of 72 hybrids, 20 cross combinations manifested significant and positive heterosis for panicle length. The estimates ranged between -29.26 (TGMS-14 × UPRI-93-287R) to 40.50 (TGMS-17 × Pant Basmati-1). The best hybrids showing high heterosis for more spikeletes number per panicle were TGMS-17 × Pant Basmati-1 (40.50), TGMS-17 × Pant Dhan-4 (37.79), TGMS-18 × Pant Dhan-4 (33.33), TGMS-10 × Pant Dhan-4 (30.81) and TGMS-1 × Pant Dhan-4 (27.13). Similar results have been reported by **Radhidevi *et al.* (2002)**, **Patil *et al.* (2003)** and **Chandirakala and Thiyagarajan (2010)**.

4.2.6 Grain number per panicle

The best check for more grain number per panicle was Pant Sankar Dhan-3, out of 72 cross combinations the 15 crosses showed the significant and positive heterosis for grain number per panicle (Table-4.9). The estimates heterosis ranged between the -31.89 (TGMS-14 × Pant Dhan-12) to 28.20 (TGMS-10 × Pant Dhan-4). The five best hybrids showing high heterosis for more grain number per panicle were TGMS-10 × Pant Dhan-4 (28.20), TGMS-17 × Pant Basmati-1 (24.09), TGMS-11 × Pant Dhan-4 (15.55), TGMS-9 × Pant Dhan-4 (15.34) and TGMS-13 × Pant Dhan-4 (15.13). Standard heterosis for this trait has also been observed by **Liu *et al.* (2005)**.

Table 4.9: Percent standard heterosis for spikelet number per panicle and grain number per panicle

Cross	Spikeletes number per panicle				Grain number per panicle			
	Pant Sankar Dhan-1	Pant Sankar Dhan-3	UPRH-128	UPRH-131	Pant Sankar Dhan-1	Pant Sankar Dhan-3	UPRH-128	UPRH-131
TGMS-1*Pant Basmati-1	-32.84**	-20.35**	-31.56**	-39.16**	-36.85**	-31.15**	-31.19**	-34.64**
TGMS-1*Pant Dhan-4	7.19**	27.13**	9.24**	-2.89	-10.06**	-1.95	-2.00	-6.91**
TGMS-1*Pant Dhan-12	-13.15**	3.00	-11.49**	-21.32**	-10.25**	-2.16	-2.21	-7.11**
TGMS-1*UPRI-93-287R	-19.53**	-4.56*	-17.99**	-27.09**	-6.96**	1.42	1.37	-3.71
TGMS-2*Pant Basmati-1	-20.42**	-5.62**	-18.90**	-27.91**	-15.57**	-7.96**	-8.01**	-12.61**
TGMS-2*Pant Dhan-4	-3.19	14.82**	-1.33	-12.29**	-4.06*	4.59*	4.53*	-0.70
TGMS-2*Pant Dhan-12	-30.64**	-17.73**	-29.31**	-37.16**	-29.21**	-22.83**	-22.87**	-26.73**
TGMS-2*UPRI-93-287R	-24.67**	-10.66**	-23.23**	-31.75**	-14.80**	-7.12**	-7.17**	-11.81**
TGMS-3*Pant Basmati-1	-8.17**	8.91**	-6.41**	-16.80**	-9.58**	-1.42	-1.48	-6.41**
TGMS-3*Pant Dhan-4	-6.29**	11.14**	-4.50*	-15.10**	2.22	11.44**	11.38**	5.81**
TGMS-3*Pant Dhan-12	-13.64**	2.42	-11.99**	-21.76**	-11.02**	-3.00	-3.06	-7.91**

TGMS-3*UPRI-93-287R	-29.58**	-16.47**	-28.23**	-36.20**	-23.41**	-16.50**	-16.55**	-20.72**
TGMS-4*Pant Basmati-1	-21.32**	-6.69**	-19.82**	-28.72**	-15.67**	-8.07**	-8.12**	-12.72**
TGMS-4*Pant Dhan-4	-16.91**	-1.46	-15.32**	-24.72**	-11.61**	-3.64	-3.69	-8.51**
TGMS-4*Pant Dhan-12	-28.11**	-14.73**	-26.73**	-34.86**	-23.12**	-16.19**	-16.23**	-20.42**
TGMS-4*UPRI-93-287R	-24.75**	-10.76**	-23.31**	-31.83**	-29.60**	-23.25**	-23.29**	-27.13**
TGMS-5*Pant Basmati-1	-26.23**	-12.50**	-24.81**	-33.16**	-17.80**	-10.38**	-10.43**	-14.92**
TGMS-5*Pant Dhan-4	-17.48**	-2.13	-15.90**	-25.24**	-16.15**	-8.59**	-8.64**	-13.21**
TGMS-5*Pant Dhan-12	-33.91**	-21.61**	-32.64**	-40.12**	-36.94**	-31.26**	-31.30**	-34.74**
TGMS-5*UPRI-93-287R	-31.13**	-18.31**	-29.81**	-37.60**	-25.92**	-19.24**	-19.28**	-23.32**
TGMS-6*Pant Basmati-1	-26.72**	-13.08**	-25.31**	-33.60**	-21.66**	-14.60**	-14.65**	-18.92**
TGMS-6*Pant Dhan-4	-5.23**	12.40**	-3.41	-14.14**	-0.97	7.96**	7.90**	2.50
TGMS-6*Pant Dhan-12	-35.86**	-23.93**	-34.64**	-41.89**	-32.69**	-26.62**	-26.66**	-30.33**
TGMS-6*UPRI-93-287R	-4.08*	13.76**	-2.25	-13.10**	-1.07	7.85**	7.79**	2.40
TGMS-7*Pant Basmati-1	-19.93**	-5.04*	-18.40**	-27.46**	-13.44**	-5.64**	-5.69**	-10.41**
TGMS-7*Pant Dhan-4	-11.19**	5.33*	-9.49**	-19.54**	-4.45*	4.16*	4.11*	-1.10
TGMS-7*Pant Dhan-12	-30.80**	-17.93**	-29.48**	-37.31**	-25.63**	-18.92**	-18.97**	-23.02**
TGMS-7*UPRI-93-287R	-32.92**	-20.45**	-31.64**	-39.23**	-33.46**	-27.46**	-27.50**	-31.13**
TGMS-8*Pant Basmati-1	-31.29**	-18.51**	-29.98**	-37.75**	-24.57**	-17.76**	-17.81**	-21.92**
TGMS-8*Pant Dhan-4	-15.03**	0.77	-13.41**	-23.02**	-18.57**	-11.23**	-11.28**	-15.72**
TGMS-8*Pant Dhan-12	-34.97**	-22.87**	-33.72**	-41.08**	-27.37**	-20.82**	-20.87**	-24.83**
TGMS-8*UPRI-93-287R	-32.03**	-19.38**	-30.72**	-38.42**	-23.41**	-16.50**	-16.55**	-20.72**
TGMS-9*Pant Basmati-1	-5.80**	11.72**	-4.00*	-14.66**	1.93	11.12**	11.06**	5.50**
TGMS-9*Pant Dhan-4	5.23**	24.80**	7.24**	-4.66**	5.80**	15.34**	15.28**	9.51**
TGMS-9*Pant Dhan-12	-20.34**	-5.52**	-18.82**	-27.83**	-17.12**	-9.65**	-9.70**	-14.22**
TGMS-9*UPRI-93-287R	-25.82**	-12.02**	-24.40**	-32.79**	-19.44**	-12.18**	-12.22**	-16.62**
TGMS-10*Pant Basmati-1	-24.84**	-10.85**	-23.40**	-31.90**	-16.44**	-8.91**	-8.96**	-13.51**
TGMS-10*Pant Dhan-4	10.29**	30.81**	12.41**	-0.07	17.60**	28.20**	28.13**	21.72**
TGMS-10*Pant Dhan-12	-38.32**	-26.84**	-37.14**	-44.12**	-33.66**	-27.68**	-27.71**	-31.33**
TGMS-10*UPRI-93-287R	-30.15**	-17.15**	-28.81**	-36.71**	-20.12**	-12.92**	-12.96**	-17.32**
TGMS-11*Pant Basmati-1	-14.62**	1.26	-12.99**	-22.65**	-13.15**	-5.32**	-5.38**	-10.11**
TGMS-11*Pant Dhan-4	6.05**	25.77**	8.08**	-3.92*	5.99**	15.55**	15.49**	9.71**
TGMS-11*Pant Dhan-12	-21.98**	-7.46**	-20.48**	-29.31**	-22.92**	-15.97**	-16.02**	-20.22**
TGMS-11*UPRI-93-287R	-27.94**	-14.54**	-26.56**	-34.72**	-21.28**	-14.18**	-14.23**	-18.52**
TGMS-12*Pant Basmati-1	-17.73**	-2.43	-16.15**	-25.46**	-21.47**	-14.39**	-14.44**	-18.72**
TGMS-12*Pant Dhan-4	4.41*	23.84**	6.41**	-5.40**	0.96	10.07**	10.01**	4.50*
TGMS-12*Pant Dhan-12	-20.18**	-5.33*	-18.65**	-27.68**	-18.86**	-11.54**	-11.59**	-16.02**
TGMS-12*UPRI-93-287R	-22.14**	-7.65**	-20.65**	-29.46**	-20.70**	-13.55**	-13.59**	-17.92**
TGMS-13*Pant Basmati-1	-23.20**	-8.92**	-21.73**	-30.42**	-28.43**	-21.98**	-22.02**	-25.93**
TGMS-13*Pant Dhan-4	4.82**	24.32**	6.83**	-5.03**	5.61**	15.13**	15.06**	9.31**
TGMS-13*Pant Dhan-12	-29.08**	-15.89**	-27.73**	-35.75**	-23.31**	-16.40**	-16.44**	-20.62**
TGMS-13*UPRI-93-287R	-17.81**	-2.52	-16.24**	-25.54**	-16.64**	-9.12**	-9.17**	-13.72**
TGMS-14*Pant Basmati-1	-31.13**	-18.32**	-29.81**	-37.60**	-28.34**	-21.88**	-21.92**	-25.83**
TGMS-14*Pant Dhan-4	-14.71**	1.16	-13.07**	-22.72**	-5.51**	3.00	2.95	-2.20
TGMS-14*Pant Dhan-12	-35.95**	-24.03**	-34.72**	-41.97**	-37.53**	-31.89**	-31.93**	-35.34**
TGMS-14*UPRI-93-287R	-40.36**	-29.26**	-39.22**	-45.97**	-34.62**	-28.73**	-28.77**	-32.33**
TGMS-15*Pant Basmati-1	-0.65	17.83**	1.25	-9.99**	-6.38**	2.06	2.00	-3.10
TGMS-15*Pant Dhan-4	-12.01**	4.36*	-10.32**	-20.28**	-12.96**	-5.11*	-5.16*	-9.91**
TGMS-15*Pant Dhan-12	-17.40**	-2.04	-15.82**	-25.17**	-18.38**	-11.02**	-11.07**	-15.52**
TGMS-15*UPRI-93-287R	-2.53	15.60**	-0.67	-11.70**	-2.52	6.27**	6.22**	0.90
TGMS-16*Pant Basmati-1	-35.86**	-23.93**	-34.64**	-41.89**	-35.30**	-29.47**	-29.51**	-33.03**
TGMS-16*Pant Dhan-4	-35.46**	-23.45**	-34.22**	-41.52**	-32.59**	-26.52**	-26.56**	-30.23**
TGMS-16*Pant Dhan-12	-21.24**	-6.59**	-19.73**	-28.65**	-20.60**	-13.44**	-13.49**	-17.82**
TGMS-16*UPRI-93-287R	-22.39**	-7.95**	-20.90**	-29.68**	-12.28**	-4.38*	-4.43*	-9.21**
TGMS-17*Pant Basmati-1	18.46**	40.50**	20.73**	7.33**	13.83**	24.09**	24.02**	17.82**
TGMS-17*Pant Dhan-4	16.18**	37.79**	18.40**	5.26**	2.61	11.86**	11.80**	6.20**
TGMS-17*Pant Dhan-12	-12.25**	4.07	-10.57**	-20.50**	-9.58**	-1.42	-1.48	-6.41**
TGMS-17*UPRI-93-287R	-14.87**	0.97	-13.24**	-22.87**	-13.25**	-5.43**	-5.48**	-10.21**
TGMS-18*Pant Basmati-1	-13.56**	2.52	-11.91**	-21.69**	-11.90**	-3.95	-4.01*	-8.81**
TGMS-18*Pant Dhan-4	12.42**	33.33**	14.57**	1.85	-1.45	7.43**	7.37**	2.00
TGMS-18*Pant Dhan-12	-7.92**	9.20**	-6.16**	-16.58**	-13.73**	-5.96**	-6.01**	-10.71**
TGMS-18*UPRI-93-287R	-16.34**	-0.78	-14.74**	-24.21**	-8.13**	0.16	0.10	-4.91*

4.2.7 Percent spikelet fertility

The best check for more percent spikeletes fertility was UPRH-131, out of 72 cross combinations the 69 crosses showed the significant and positive heterosis for percent spikelet fertility. The estimates heterosis ranged between the -4.14 (TGMS-1 × Pant Dhan-4) to 32.08 (TGMS-1 × UPRI-93-287R). The five best hybrids showed high heterosis for more percent spikelet fertility were TGMS-1 × UPRI-93-287R (32.08), TGMS-10 × UPRI-93-287R (30.65), TGMS-2 × UPRI-93-287R (29.23), TGMS-16 × UPRI-93-287R (29.11) and TGMS-8 × UPRI-93-287R (28.74).

4.2.8 1000 grain weight

The best check for 1000 grain weight was Pant Sankar Dhan-1. Out of 72 hybrids, 33 cross combinations manifested significant and positive heterosis for 1000 grain weight. The estimates ranged between -14.75 (TGMS-7 × Pant Basmati-1) to 26.70 (TGMS-4 × Pant Dhan-4). The best hybrids showing high heterosis for more 1000 grain weight over the check were TGMS-4 × Pant Dhan-4 (26.70), TGMS-18 × Pant Dhan-4 (24.04), TGMS-15 × Pant Dhan-4 (21.56), TGMS-3 × Pant Dhan-4 (14.85) and TGMS-14 × UPRI-93-287R (13.77). Significant positive heterosis for 1000 grain weight have also been reported by Ramalingam et al. (2001), Suresh et al. (2000), Liu et al. (2005) and Chandirakala and Thiagarajan (2010).

Table 4.10: Percent standard heterosis percent spikeletes fertility and 1000 grain weight

Cross	Percent Spikeletes fertility				1000 Grain weight			
	Pant Sankar Dhan-1	Pant Sankar Dhan-3	UPRH-128	UPRH-131	Pant Sankar Dhan-1	Pant Sankar Dhan-3	UPRH-128	UPRH-131
TGMS-1*Pant Basmati-1	-5.95**	-13.56**	0.54	7.44**	-6.37**	-9.96**	-18.71**	-9.16**
TGMS-1*Pant Dhan-4	-16.09**	-22.87**	-10.29**	-4.14	8.63**	4.46*	-5.68**	5.39*
TGMS-1*Pant Dhan-12	3.35	-5.01*	10.49**	18.07**	6.97**	2.87	-7.12**	3.78
TGMS-1*UPRI-93-287R	15.61**	6.26**	23.60**	32.08**	9.21**	5.02*	-5.18**	5.95**
TGMS-2*Pant Basmati-1	6.11**	-2.47	13.44**	21.22**	-10.33**	-13.77**	-22.15**	-13.01**
TGMS-2*Pant Dhan-4	-0.90	-8.91**	5.94*	13.21**	6.56**	2.47	-7.48**	3.38
TGMS-2*Pant Dhan-12	2.06	-6.19**	9.11**	16.60**	4.72*	0.70	-9.08**	1.59
TGMS-2*UPRI-93-287R	13.12**	3.97*	20.93**	29.23**	8.22**	4.06	-6.04**	4.98*
TGMS-3*Pant Basmati-1	-1.52	-9.48**	5.28*	12.50**	6.81**	2.71	-7.27**	3.62
TGMS-3*Pant Dhan-4	9.09**	0.27	16.63**	24.63**	14.85**	10.44**	-0.29	11.41**
TGMS-3*Pant Dhan-12	3.03	-5.30**	10.15**	17.70**	-5.79*	-9.40**	-18.20**	-8.60**
TGMS-3*UPRI-93-287R	8.76**	-0.03	16.28**	24.25**	-0.57	-4.38*	-13.67**	-3.54
TGMS-4*Pant Basmati-1	7.20**	-1.47	14.61**	22.47**	-11.68**	-15.07**	-23.32**	-14.32**
TGMS-4*Pant Dhan-4	6.39**	-2.21	13.74**	21.54**	26.70**	21.83**	10.00**	22.91**
TGMS-4*Pant Dhan-12	6.95**	-1.69	14.34**	22.18**	14.78**	10.37**	-0.35	11.35**
TGMS-4*UPRI-93-287R	-6.42**	-13.99**	0.04	6.90**	-4.54*	-8.21**	-17.12**	-7.40**
TGMS-5*Pant Basmati-1	11.44**	2.43	19.13**	27.31**	-0.90	-4.70*	-13.96**	-3.86
TGMS-5*Pant Dhan-4	1.62	-6.60**	8.64**	16.09**	10.79**	6.53**	-3.81	7.48**
TGMS-5*Pant Dhan-12	-4.59*	-12.31**	2.00	8.99**	14.68**	10.28**	-0.43	11.25**

TGMS-5*UPRI-93-287R	7.56**	-1.13	14.99**	22.88**	-0.06	-3.89	-13.23**	-3.04
TGMS-6*Pant Basmati-1	6.90**	-1.74	14.28**	22.13**	-1.48	-5.26*	-14.46**	-4.42*
TGMS-6*Pant Dhan-4	4.50*	-3.95*	11.72**	19.38**	5.22*	1.18	-8.65**	2.08
TGMS-6*Pant Dhan-12	4.96*	-3.52	12.21**	19.91**	8.88**	4.70*	-5.47**	5.63*
TGMS-6*UPRI-93-287R	3.15	-5.19**	10.27**	17.83**	-4.71*	-8.37**	-17.27**	-7.56**
TGMS-7*Pant Basmati-1	8.11**	-0.63	15.58**	23.51**	-14.75**	-18.02**	-25.98**	-17.30**
TGMS-7*Pant Dhan-4	7.60**	-1.10	15.04**	22.93**	6.89**	2.79	-7.19**	3.70
TGMS-7*Pant Dhan-12	7.48**	-1.21	14.90**	22.79**	-4.97*	-8.62**	-17.49**	-7.81**
TGMS-7*UPRI-93-287R	-0.80	-8.82**	6.05**	13.33**	-1.22	-5.01*	-14.23**	-4.17
TGMS-8*Pant Basmati-1	9.79**	0.91	17.37**	25.43**	-0.58	-4.40*	-13.68**	-3.55
TGMS-8*Pant Dhan-4	-4.16	-11.90**	2.46	9.49**	0.69	-3.17	-12.58**	-2.32
TGMS-8*Pant Dhan-12	11.68**	2.66	19.40**	27.59**	7.72**	3.59	-6.47**	4.50*
TGMS-8*UPRI-93-287R	12.69**	3.58	20.47**	28.74**	3.83	-0.16	-9.86**	0.72
TGMS-9*Pant Basmati-1	8.22**	-0.53	15.69**	23.63**	2.09	-1.83	-11.37**	-0.96
TGMS-9*Pant Dhan-4	0.54	-7.58**	7.49**	14.86**	10.70**	6.45**	-3.88*	7.40**
TGMS-9*Pant Dhan-12	4.05	-4.36*	11.23**	18.87**	0.93	-2.95	-12.37**	-2.09
TGMS-9*UPRI-93-287R	8.61**	-0.17	16.11**	24.08**	3.49	-0.48	-10.14**	0.40
TGMS-10*Pant Basmati-1	11.18**	2.19	18.85**	27.01**	-8.27**	-11.79**	-20.36**	-11.01**
TGMS-10*Pant Dhan-4	6.63**	-1.99	13.99**	21.81**	4.25	0.25	-9.48**	1.14
TGMS-10*Pant Dhan-12	7.56**	-1.13	14.99**	22.88**	2.09	-1.83	-11.37**	-0.96
TGMS-10*UPRI-93-287R	14.36**	5.12*	22.26**	30.65**	6.15**	2.07	-7.84**	2.97
TGMS-11*Pant Basmati-1	1.72	-6.50**	8.75**	16.21**	-7.79**	-11.33**	-19.94**	-10.54**
TGMS-11*Pant Dhan-4	-0.05	-8.13**	6.86**	14.19**	11.35**	7.08**	-3.32	8.03**
TGMS-11*Pant Dhan-12	-1.21	-9.19**	5.62*	12.86**	-7.28**	-10.84**	-19.50**	-10.05**
TGMS-11*UPRI-93-287R	9.26**	0.42	16.80**	24.82**	-0.39	-4.21	-13.51**	-3.36
TGMS-12*Pant Basmati-1	-4.55*	-12.26**	2.05	9.05**	-4.14	-7.82**	-16.77**	-7.01**
TGMS-12*Pant Dhan-4	-3.29	-11.11**	3.39	10.48**	12.68**	8.35**	-2.17	9.31**
TGMS-12*Pant Dhan-12	1.66	-6.56**	8.68**	16.13**	-2.39	-6.14**	-15.25**	-5.31*
TGMS-12*UPRI-93-287R	1.86	-6.38**	8.89**	16.36**	8.22**	4.06	-6.04**	4.98*
TGMS-13*Pant Basmati-1	-6.81**	-14.35**	-0.38	6.46**	-9.27**	-12.75**	-21.22**	-11.98**
TGMS-13*Pant Dhan-4	0.75	-7.39**	7.71**	15.10**	-1.23	-5.02*	-14.24**	-4.18
TGMS-13*Pant Dhan-12	8.15**	-0.59	15.62**	23.55**	3.00	-0.96	-10.58**	-0.08
TGMS-13*UPRI-93-287R	1.43	-6.77**	8.43**	15.87**	1.59	-2.31	-11.80**	-1.45
TGMS-14*Pant Basmati-1	4.06	-4.35*	11.25**	18.88**	5.50*	1.45	-8.41**	2.34
TGMS-14*Pant Dhan-4	10.78**	1.83	18.44**	26.56**	7.90**	3.76	-6.32**	4.68*
TGMS-14*Pant Dhan-12	-2.46	-10.35**	4.27	11.43**	7.22**	3.11	-6.91**	4.02
TGMS-14*UPRI-93-287R	9.62**	0.76	17.20**	25.24**	13.77**	9.40**	-1.22	10.37**
TGMS-15*Pant Basmati-1	-5.76**	-13.38**	0.75	7.66**	8.80**	4.62*	-5.54**	5.55*
TGMS-15*Pant Dhan-4	-1.08	-9.08**	5.75*	13.01**	21.56**	16.89**	5.54**	17.93**
TGMS-15*Pant Dhan-12	-1.17	-9.16**	5.65*	12.90**	-12.01**	-15.39**	-23.61**	-14.64**
TGMS-15*UPRI-93-287R	0.02	-8.06**	6.93**	14.27**	-2.54	-6.28**	-15.38**	-5.45*
TGMS-16*Pant Basmati-1	0.89	-7.27**	7.86**	15.26**	-1.39	-5.18*	-14.39**	-4.34*
TGMS-16*Pant Dhan-4	4.44*	-4.00*	11.65**	19.31**	8.70**	4.53*	-5.62**	5.45*
TGMS-16*Pant Dhan-12	0.82	-7.33**	7.78**	15.17**	1.75	-2.15	-11.65**	-1.29
TGMS-16*UPRI-93-287R	13.02**	3.88	20.82**	29.11**	9.36**	5.17*	-5.05*	6.10**
TGMS-17*Pant Basmati-1	-3.90	-11.67**	2.73	9.78**	2.75	-1.20	-10.79**	-0.32
TGMS-17*Pant Dhan-4	-11.67**	-18.81**	-5.57*	0.91	11.30**	7.03**	-3.37	7.97**
TGMS-17*Pant Dhan-12	3.05	-5.28**	10.17**	17.73**	0.47	-3.39	-12.77**	-2.53
TGMS-17*UPRI-93-287R	1.88	-6.35**	8.92**	16.39**	-5.62*	-9.24**	-18.06**	-8.44**
TGMS-18*Pant Basmati-1	1.93	-6.31**	8.97**	16.45**	7.14**	3.03	-6.98**	3.94
TGMS-18*Pant Dhan-4	-12.34**	-19.42**	-6.28**	0.15	24.04**	19.28**	7.70**	20.34**
TGMS-18*Pant Dhan-12	-6.31**	-13.88**	0.16	7.03**	-0.47	-4.29*	-13.59**	-3.44
TGMS-18*UPRI-93-287R	9.83**	0.95	17.41**	25.47**	4.47*	0.46	-9.29**	1.35

4.2.9 Harvest index

The best check for high harvest index was Pant Sankar Dhan-1, out of 72 cross combinations the 34 crosses showed the significant and positive heterosis for Harvest index. The estimates heterosis ranged between the -35.22 (TGMS-15 × Pant Basmati-1) to

36.98 (TGMS-16 × Pant Basmati-1). The five best hybrids showing high heterosis for high Harvest index were TGMS-16 × Pant Basmati-1 (36.98), TGMS-13 × Pant Basmati-1 (28.91), TGMS-10 × Pant Dhan-4 (27.20), TGMS-18 × Pant Dhan-4 (25.53) and TGMS-10 × Pant Dhan-12 (23.17).

4.2.10 Grain yield per plant

The best check for grain yield per plant was UPRH-131. Out of 72 hybrids, 71 cross combinations manifested significant and positive heterosis for grain yield per plant (Table-4.11). The estimates ranged between 7.56 (TGMS-3 × UPRI-93-287R) to 184.11 (TGMS-14 × Pant Dhan-4). The best hybrids showing high heterosis for more grain yield per plant over the check were TGMS-14 × Pant Dhan-4 (184.11), TGMS-16 × UPRI-93-287R (157.90), TGMS-17 × UPRI-93-287R (156.52), TGMS-9 × Pant Basmati-1 (146.86) and TGMS-14 × UPRI-93-287R (128.94). Heterosis for grain yield over standard parent has also been observed by **Dela-Rosa *et al.* (2003)**.

Table 4.11: Percent standard heterosis harvest index and grain yield per plant

Cross	Harvest index				Grain yield per plant			
	Pant Sankar Dhan-1	Pant Sankar Dhan-3	UPRH-128	UPRH-131	Pant Sankar Dhan-1	Pant Sankar Dhan-3	UPRH-128	UPRH-131
TGMS-1*Pant Basmati-1	-13.78**	-37.72**	-17.63**	-27.89**	-1.20	-4.83	-6.68*	15.84**
TGMS-1*Pant Dhan-4	-24.77**	-45.65**	-28.12**	-37.08**	8.22*	4.24	2.21	26.88**
TGMS-1*Pant Dhan-12	11.09**	-19.75**	6.13**	-7.09**	5.88	1.98	0.00	24.14**
TGMS-1*UPRI-93-287R	-3.06	-29.97**	-7.38**	-18.92**	9.41**	5.38	3.33	28.27**
TGMS-2*Pant Basmati-1	0.11	-27.68**	-4.35*	-16.27**	22.33**	17.83**	15.54**	43.42**
TGMS-2*Pant Dhan-4	3.54	-25.20**	-1.08	-13.40**	3.52	-0.29	-2.23	21.37**
TGMS-2*Pant Dhan-12	2.26	-26.13**	-2.30	-14.47**	8.23*	4.25	2.22	26.89**
TGMS-2*UPRI-93-287R	11.08**	-19.75**	6.13**	-7.10**	5.87	1.97	-0.01	24.12**
TGMS-3*Pant Basmati-1	7.22**	-22.54**	2.44	-10.32**	14.11**	9.90**	7.77*	33.78**
TGMS-3*Pant Dhan-4	18.76**	-14.21**	13.46**	-0.67	21.16**	16.70**	14.43**	42.05**
TGMS-3*Pant Dhan-12	13.39**	-18.09**	8.33**	-5.17**	19.98**	15.56**	13.31**	40.66**
TGMS-3*UPRI-93-287R	-0.04	-27.79**	-4.50*	-16.40**	-8.25*	-11.63**	-13.35**	7.56
TGMS-4*Pant Basmati-1	8.42**	-21.68**	3.58	-9.32**	34.69**	29.73**	27.21**	57.91**
TGMS-4*Pant Dhan-4	-13.22**	-37.31**	-17.09**	-27.42**	29.40**	24.63**	22.21**	51.71**
TGMS-4*Pant Dhan-12	-6.93**	-32.76**	-11.08**	-22.16**	16.46**	12.17**	9.99**	36.54**
TGMS-4*UPRI-93-287R	-12.37**	-36.70**	-16.28**	-26.71**	47.04**	41.63**	38.88**	72.39**
TGMS-5*Pant Basmati-1	2.37	-26.04**	-2.19	-14.38**	18.22**	13.87**	11.66**	38.60**
TGMS-5*Pant Dhan-4	-4.21	-30.80**	-8.48**	-19.88**	52.94**	47.30**	44.44**	79.30**
TGMS-5*Pant Dhan-12	3.46	-25.26**	-1.15	-13.47**	5.87	1.97	-0.01	24.12**
TGMS-5*UPRI-93-287R	0.61	-27.32**	-3.88	-15.85**	43.51**	38.22**	35.53**	68.25**
TGMS-6*Pant Basmati-1	-2.66	-29.68**	-7.00**	-18.58**	32.34**	27.47**	24.99**	55.15**
TGMS-6*Pant Dhan-4	19.13**	-13.94**	13.82**	-0.36	77.63**	71.09**	67.76**	108.25**
TGMS-6*Pant Dhan-12	7.93**	-22.03**	3.11	-9.73**	47.04**	41.63**	38.88**	72.39**
TGMS-6*UPRI-93-287R	-11.09**	-35.77**	-15.05**	-25.64**	16.47**	12.18**	10.00**	36.55**
TGMS-7*Pant Basmati-1	3.53	-25.21**	-1.09	-13.41**	45.86**	40.49**	37.76**	71.00**
TGMS-7*Pant Dhan-4	18.30**	-14.54**	13.02**	-1.06	61.16**	55.23**	52.21**	88.94**
TGMS-7*Pant Dhan-12	-12.54**	-36.81**	-16.44**	-26.85**	17.64**	13.30**	11.10**	37.92**
TGMS-7*UPRI-93-287R	1.29	-26.82**	-3.22	-15.28**	69.39**	63.15**	59.98**	98.59**
TGMS-8*Pant Basmati-1	11.45**	-19.49**	6.48**	-6.79**	32.92**	28.02**	25.53**	55.83**
TGMS-8*Pant Dhan-4	-6.63**	-32.55**	-10.80**	-21.91**	36.46**	31.43**	28.88**	59.98**

TGMS-8*Pant Dhan-12	8.63**	-21.52**	3.79	-9.14**	35.28**	30.30**	27.77**	58.60**
TGMS-8*UPRI-93-287R	-1.69	-28.98**	-6.07**	-17.78**	27.05**	22.37**	19.99**	48.95**
TGMS-9*Pant Basmati-1	10.80**	-19.95**	5.86**	-7.33**	110.56**	102.80**	98.86**	146.86**
TGMS-9*Pant Dhan-4	-8.93**	-34.21**	-13.00**	-23.83**	26.46**	21.80**	19.43**	48.26**
TGMS-9*Pant Dhan-12	0.85	-27.15**	-3.65	-15.66**	16.45**	12.16**	9.98**	36.52**
TGMS-9*UPRI-93-287R	-2.33	-29.44**	-6.69**	-18.31**	37.63**	32.57**	29.99**	61.36**
TGMS-10*Pant Basmati-1	18.93**	-14.08**	13.63**	-0.53	56.46**	50.69**	47.76**	83.43**
TGMS-10*Pant Dhan-4	27.20**	-8.11**	21.53**	6.39**	65.87**	59.76**	56.65**	94.46**
TGMS-10*Pant Dhan-12	23.17**	-11.02**	17.68**	3.02	41.16**	35.96**	33.32**	65.50**
TGMS-10*UPRI-93-287R	6.80**	-22.85**	2.04	-10.67**	51.75**	46.16**	43.32**	77.91**
TGMS-11*Pant Basmati-1	3.94	-24.91**	-0.69	-13.06**	45.87**	40.50**	37.77**	71.01**
TGMS-11*Pant Dhan-4	-4.99*	-31.36**	-9.23**	-20.54**	35.29**	30.31**	27.78**	58.61**
TGMS-11*Pant Dhan-12	-17.69**	-40.53**	-21.36**	-31.15**	20.58**	16.14**	13.88**	41.36**
TGMS-11*UPRI-93-287R	9.86**	-20.64**	4.96*	-8.12**	81.17**	74.50**	71.10**	112.40**
TGMS-12*Pant Basmati-1	-0.58	-28.18**	-5.02*	-16.85**	48.21**	42.75**	39.98**	73.76**
TGMS-12*Pant Dhan-4	1.38	-26.76**	-3.14	-15.21**	50.57**	45.03**	42.21**	76.53**
TGMS-12*Pant Dhan-12	-10.58**	-35.40**	-14.57**	-25.21**	29.40**	24.63**	22.21**	51.71**
TGMS-12*UPRI-93-287R	2.83	-25.71**	-1.76	-14.00**	62.34**	56.36**	53.32**	90.32**
TGMS-13*Pant Basmati-1	28.91**	-6.87**	23.16**	7.82**	49.99**	44.46**	41.65**	75.84**
TGMS-13*Pant Dhan-4	20.72**	-12.79**	15.34**	0.97	34.10**	29.17**	26.65**	57.22**
TGMS-13*Pant Dhan-12	16.01**	-16.19**	10.84**	-2.97	68.22**	62.02**	58.87**	97.22**
TGMS-13*UPRI-93-287R	8.05**	-21.94**	3.23	-9.63**	53.51**	47.86**	44.99**	79.98**
TGMS-14*Pant Basmati-1	0.61	-27.32**	-3.88	-15.85**	25.87**	21.24**	18.88**	47.57**
TGMS-14*Pant Dhan-4	20.49**	-12.96**	15.12**	0.77	142.33**	133.41**	128.87**	184.11**
TGMS-14*Pant Dhan-12	7.48**	-22.35**	2.69	-10.10**	37.63**	32.57**	29.99**	61.36**
TGMS-14*UPRI-93-287R	10.21**	-20.38**	5.30*	-7.82**	95.27**	88.08**	84.43**	128.94**
TGMS-15*Pant Basmati-1	-35.22**	-53.20**	-38.11**	-45.82**	11.75**	7.64*	5.55	31.02**
TGMS-15*Pant Dhan-4	9.15**	-21.15**	4.28	-8.71**	57.63**	51.83**	48.88**	84.81**
TGMS-15*Pant Dhan-12	3.55	-25.19**	-1.06	-13.39**	23.53**	18.98**	16.67**	44.82**
TGMS-15*UPRI-93-287R	-8.73**	-34.07**	-12.80**	-23.67**	32.35**	27.48**	25.00**	55.17**
TGMS-16*Pant Basmati-1	36.98**	-1.04	30.87**	14.57**	88.23**	81.30**	77.77**	120.68**
TGMS-16*Pant Dhan-4	9.48**	-20.91**	4.59*	-8.44**	37.63**	32.57**	29.99**	61.36**
TGMS-16*Pant Dhan-12	0.18	-27.63**	-4.29	-16.21**	45.87**	40.50**	37.77**	71.01**
TGMS-16*UPRI-93-287R	8.73**	-21.45**	3.88	-9.06**	119.98**	111.88**	107.76**	157.90**
TGMS-17*Pant Basmati-1	9.09**	-21.19**	4.22	-8.76**	81.17**	74.50**	71.10**	112.40**
TGMS-17*Pant Dhan-4	10.62**	-20.08**	5.69*	-7.48**	62.35**	56.37**	53.33**	90.33**
TGMS-17*Pant Dhan-12	15.92**	-16.26**	10.75**	-3.05	64.69**	58.63**	55.54**	93.08**
TGMS-17*UPRI-93-287R	10.06**	-20.49**	5.15*	-7.95**	118.80**	110.74**	106.65**	156.52**
TGMS-18*Pant Basmati-1	0.60	-27.32**	-3.88	-15.86**	48.22**	42.76**	39.99**	73.77**
TGMS-18*Pant Dhan-4	25.53**	-9.31**	19.93**	4.99*	71.74**	65.41**	62.20**	101.34**
TGMS-18*Pant Dhan-12	4.76*	-24.32**	0.09	-12.38**	91.75**	84.69**	81.09**	124.80**
TGMS-18*UPRI-93-287R	3.00	-25.59**	-1.59	-13.85**	76.46**	69.97**	66.66**	106.88**

*Significant at p = 0.05, **Significant at p = 0.01

Table 4.12: Top ranking five economic crosses for seed yield per plant and their performance in relation to other parameters

S.NO.	Cross combinations	Standard heterosis	SCA effect	per se performance	Magnitude of GCA effect	
					P ₁	P ₂
1.	TGMS-14 × Pant Dhan-4	184.11	21.28**	82.40	H	H
2.	TGMS-16 × UPRI-93-287R	157.90	13.24**	74.80	H	H
3.	TGMS-17 × UPRI-93-287R	156.52	9.84**	74.40	H	H
4.	TGMS-9 × Pant Basmati-1	146.86	21.88**	71.60	H	L
5.	TGMS-14 × UPRI-93-287R	128.94	4.04**	66.40	H	H

*Significant at p = 0.05, **Significant at p = 0.01

Based on the significant and high estimates of standard heterosis five crosses for seed yield were identified which also showed high SCA effect and *per se* performance. All the five crosses emerged from the high $\times$ high GCA effect parents except one cross which had high $\times$ low GCA effect parents. These hybrids can be used as hybrids in future for economic exploitation of multiplication testing. Positive standard heterosis with significant SCA effect for different traits have also been reported by **Tiwari *et al.*, (2011)** and **Divyapriya and Kalaiyarasi(2014)**. High heterosis along with high sca effect for different traits has also been observed by **Sharma *et al.* (2001)**.

Several findings have been reported by different researchers for standard heterosis in different genotypes. **Pandey *et al.* (1995)**, reported significant standard heterosis for grain yield, panicle number plant, panicle length, grains per panicle and 1000-grain weight, Sharma and **Malik (2008)** observed the maximum heterosis for grain yield, **Malarvizhi *et al.* (2009)** reported heterosis for number of productive tillers, panicle length, spikelets fertility and harvest index, **Chandiraka *et al.* (2010)** also observed significant and positive heterosis for yield contributing traits viz., number of productive tillers per plant, panicle length, and number of filled grains per panicle, spikelets fertility and 1000 grain weight, **Rahimi *et al.* (2010)** reported significant and positive heterosis over check for grain yield per plant and 1000 grain weight, **Tiwari *et al.*, (2011)** found that most of the crosses which exhibited superiority over better parent or standard variety for grain yield also showed significant heterosis for number of fertile spikelets and number of spikelets per panicle, **Ghara *et al.*, (2014)** reported high heterosis for grain yield per plant, **Lopez and Virmani (2000)** studied heterosis using TGMS lines and reported higher grain yield in some heterotic hybrids and **Saxena (2000)** finding also for grain yield heterosis.

4.3 Component of genetic variance

Estimates of genetic components of variance are presented in (Table 4.3). Estimates of variance of general combining ability (σ_{gca}^2) was higher than those of variance of specific combining ability (σ_{sca}^2) for plant height, panicle length and spikelets number per panicle, it means these traits governed by additive gene action. Other traits viz. days to 50% flowering, number of grain number of panicle, panicles number per plant, spikelets fertility, 1000 grain weight, grain yield per plant and

harvest index showing preponderance of non-additive gene action, non additive gene action for these traits have been reported by **Bagheri and Jelodar (2010)**, **Saidaiah et al. (2010)**, **Divyapriya and Kalaiyarasi (2014)** and **Ghara et al., (2014)**. It was supported by ratio of variance of general to specific combining ability ($\sigma_{gca}^2/\sigma_{sca}^2$) which was more than unity plant height, panicle length and spikelet number per panicle. These traits governed by additive gene action. The ratio of dominance to additive variance (σ_D^2/σ_A^2) indicated the degree of dominance whether the traits showed dominance, incomplete dominance and over dominance. The ratio less than unity for plant height, panicle length, spikeletes number per panicle, grain number per panicle and 1000 grain weight indicated that these traits showed incomplete dominance whereas the ratio (σ_D^2/σ_A^2)^{1/2} being greater than unity for plant height, panicle length, spikelet number per panicle, grain number per panicle and 1000 grain weight it means these traits showed over-dominance.

Therefore it appeared that the inheritance of days to 50% flowering **Panwar (2005)**, **Sharma et al. (2005)** and **Sharma (2006)** reported non-additive], is additive while emphasized the importance of additive and non-additive gene effects for days to flowering., panicle number per plant (**Ramalingam et al. 2000; Punitha et al. 2004; Sharma, 2006**), Percent Spikeletes Fertility (**Hossen et al. 2005; Gnanasekaran et al. 2006**), harvest Index (**Mehla et al. 2000; Hosseini et al. 2005; Gnanasekaran et al. 2006**) and Grain Yield per Plant (**Ramalingam et al. 2000; Veni and Rani, 2003; Sharma et al. 2005; Sharma, 2006**) were controlled by non-additive gene effects also observed by **Thakare et al., (2013)**, **Dorosti and Monajjem (2014)** and plant height (**Singh et al. 1998; Vanaja et al. 2003; Gnanasekaran et al. 2006**), panicle length (**Ramalingam et al. 2000; Radhidevi et al. 2002; Gnanasekaran et al. 2006**), spikeletes number per panicle, grain number per panicle (**Thirumeni et al. 2000; Sharma, 2006**) and 1000 grain weight (**Vanaja et al. 2003; Gnanasekaran et al. 2006; Sharma, 2006**) were controlled by additive gene action. Such type of gene action clearly indicated that pure line and heterosis breeding would be ideal for their improvement. 1000 grain weight controlled by additive gene action also reported by **Vivekanandan and Giridharan (1997)**, **Roy and Mandal (2001)** observed for panicle length and 1000 grain weight.

Table 4.13: Estimates of genetic components of rice genotypes

Parents	Days to 50% flowering	Plant height	Panicle number per plant	Panicle length	Spikelets number per panicle	Grain number per panicle	Percent spikeletes fertility	1000 grain weight	harvest index	Grain yield per plant
sl ² Line HS	24.50	6.11	1.93	0.75	438.51	190.86	8.72	0.57	16.50	57.82
sl ² Tester HS	3.00	22.91	0.23	1.40	541.41	268.28	5.41	1.71	1.01	8.04
σ_{gca}^2	6.91	19.85	0.54	1.28	522.70	254.21	6.01	1.50	3.83	17.09
σ_{sca}^2	29.48	16.61	3.86	0.79	484.24	388.73	23.32	2.70	28.68	64.90
sl ² e	1.36	2.80	0.03	0.18	9.45	7.36	1.64	0.15	0.65	0.62
sl ² a(F = 1)	13.82	39.71	1.09	2.56	1045.40	508.41	12.02	3.00	7.65	34.18
sl ² D(F = 1)	29.48	16.61	3.86	0.79	484.24	388.73	23.32	2.70	28.68	64.90
sl ² a / Var.D	0.47	2.39	0.28	3.25	2.16	1.31	0.52	1.11	0.27	0.53
Degree of Dominance (σ_D^2/σ_A^2) ^{1/2}	1.46	0.65	1.89	0.55	0.68	0.87	1.39	0.95	1.94	1.38
$\sigma_{gca}^2/\sigma_{sca}^2$	0.23	1.19	0.14	1.62	1.08	0.65	0.26	0.55	0.13	0.26
sl ² p	44.65	59.12	4.97	3.53	1539.08	904.50	36.99	5.85	36.98	99.70
Heritability (Broad Sense) %	92	81	98	82	98	97	86	90	95	98
Genetic Advance	4.26	10.64	1.00	2.81	54.89	34.82	4.07	2.56	2.59	7.05
Genetic advance in mean percentage	4.25	9.59	8.26	9.85	27.23	19.93	4.67	10.26	4.99	14.38

4.3.1 Heritability and genetic advance

There was little difference between phenotypic and genotypic variances of all parameters showing negligible effect of environment on the inheritance of these traits. Estimates of broad sense heritability were high and found significant as their absolute values exceeded twice their respective standard errors. The high heritability in broad sense for all the traits in the present study is also reported by **Rita *et al.* (2009)** and **Ukaoma *et al.* (2013)** along with genetic advance (low, moderate and high) (Table-4.13) in term of percentage of mean.

High heritability associated with low genetic advance for days to 50% flowering ($h^2=92$, GA=4.25) was observed **Mishra *et al.* (2003)** and **Agrawal (2003)** recorded high estimates of broad sense heritability along with low genetic advance for days to flowering. For plant height ($h^2=81$, GA=9.59) high heritability observed to this study was also observed for **Ghara *et al.*, (2014)**, for panicle length ($h^2=98$, GA=8.26) the results obtained are in same with results of **Rao *et al.* (1997)**, **Ali *et al.* (2000)** and **Shanthi and Singh (2002)** show was high values of broad sense heritability. High broad sense heritability and low genetic advance were observed by **Swati and Ramesh (2004)** whereas high heritability and moderate genetic advance were recorded by **Elayaraja *et al.* (2005)** in rice. For panicle number per plant ($h^2=82$, GA=9.85), per cent spikelet fertility ($h^2=86$, GA=4.67) and harvest index ($h^2=95$, GA=4.99) also showed high heritability coupled with low genetic advance. High heritability with less genetic advance for days to flowering and plant height also observed by **Paikhomba *et al.* (2014)**.

High heritability associated with moderate genetic advance for grain number per panicle ($h^2=97$, GA=19.93) also observed by **Paikhomba *et al.* (2014)**, this estimates of heritability in broad sense grain yield per plant ($h^2=98$, GA=14.38) observed have are similar to results of **Pfukrei *et al.* (2011)**, **Toshimenla and Changkija, (2013)** and **Koli *et al.* (2013)**. For 1000 grain weight ($h^2=90$, GA=10.26).

High genetic advance associated with high heritability was observed only for one trait viz. spikeletes number per panicle ($h^2=98$, GA=27.23), also reported by **Rita *et al.* (2009)** and **Singh *et al.* (2011)** who observed that maximum genetic advance as per cent of mean was recorded for number of spikelets per panicle with high value of heritability.

4.4 Characterization of TGMS (Thermosensitive Genetic Male Sterile Line)

4.4.1 Morphological characterization of TGMS lines

4.4.2 Molecular characterization of TGMS lines

4.4.1 Morphological characterization of TGMS lines

The eighteen TGMS line were evaluated for 16 different traits and based on these traits the line were identified as best line for hybrid seed production. The observations recorded on the floral and morphological characters of TGMS lines are listed in Table-4.14 and Table-4.15.

4.4.1.1 Days to 50% Flowering

For days to flowering 12 lines significant and had mean value high. The days of flowering varied from 96 days (UPRI-97-60-8) to 119 days (UPRI-99-73-2). The early flowering lines were UPRI-97-60-8 (96), UPRI-99-74-3 (108), UPRI-99-70-1 (110), UPRI-97-60-1 (110), UPRI-99-74-4 (110) and UPRI-99-75-1 (110).

4.4.1.2 Plant height

Plant height range was between the 72.30 cm. (UPRI-99-71-1) to 88.64 cm. (UPRI-99-74-1). The 11 lines showed significantly and high mean values. Lines with dwarf plant height were UPRI-99-71-1 (72.30), UPRI-99-73-4 (76.31), UPRI-99-73-3 (76.54) and UPRI-99-72-1 (78.90).

4.4.1.3 Number of effective tillers

Number of effective tillers varied from line to line with minimum tillers number between 7.5 in UPRI-99-74-3 and maximum in UPRI-99-74-4 (15.1). The ten lines showed the significantly with high mean values. Highest tillers number lines were UPRI-99-74-4 (15.1), UPRI-99-78-1 (14.5), UPRI-99-72-4 (14.2) and UPRI-99-73-2 (14.2).

4.4.1.4 Stem color

Two types of stem color observed were green and purple, most of the lines showed purple stem color above the ground stem and seven lines showing the green stem were UPRI-99-70-1, UPRI-99-71-1, UPRI-99-71-2, UPRI-99-73-1, UPRI-99-73-2, UPRI-99-73-3 and UPRI-99-78-1. The purple stem color can be used as a morphological marker for identification of these lines.

4.4.1.5 Panicle type

Intermediate and open type panicles were observed in these 18 lines. All the lines produced intermediate panicle type except for six lines viz. UPRI-99-71-2, UPRI-99-79-1, UPRI-97-60-1, UPRI-99-75-1, UPRI-99-78-1 and UPRI-97-60-8 which had open panicle type.

4.4.1.6 Panicle length

The panicle length was observed more in ten lines which showed significant mean value which range of between 25.28 (UPRI-99-72-3) to 21.08 (UPRI-99-70-1). The best five lines which had maximum length of panicle were UPRI-99-72-3 (25.28), UPRI-99-72-1 (24.10), UPRI-99-74-1 (24.04), UPRI-99-71-1 (24.00) and UPRI-99-74-3 (23.80).

4.4.1.7 Number of spikelet

The number of spikelets varied from minimum in UPRI-99-73-3 (127.6) to maximum in UPRI-99-78-1 (303.5). Eight lines showed the significantly higher mean value. The five lines observed with maximum spikelets number were UPRI-99-78-1 (303.5), UPRI-99-79-1 (296.3), UPRI-99-73-1 (239.0), UPRI-99-70-1 (236.0) and UPRI-99-73-2 (232.0).

4.4.1.8 Awning

Based on the presence and absence TGMS lines divides in to two categories, all the lines have absence of awns but five lines with awns viz. UPRI-99-70-1, UPRI-99-74-3, UPRI-99-74-1, UPRI-99-74-4 and UPRI-99-75-1.

4.4.1.9 Apiculous pigmentation

The color of Apiculous varied from purple to absent, most of the lines having purple apiculous color and seven lines have not any color viz. UPRI-99-70-1, UPRI-99-71-1, UPRI-99-71-2, UPRI-99-73-1, UPRI-99-73-2, UPRI-99-73-3 and UPRI-99-78-1. The lines which have purple stem color also have purple Apiculous. The purple Apiculous and color can be used as a morphological marker for identification of these lines.

4.4.1.10 Stigma color

The color of stigma varied from purple to white, only three lines have the white color stigmas viz. UPRI-99-71-1, UPRI-99-73-1 and UPRI-99-73-2. Other lines have purple color stigma.

Table 4.14:- Floral and morphological traits recorded in TGMS lines

Line number	TGMS name	Days to 50 % flowering	Plant height	Number of effective tillers	Stem color	Panicle type	Panicle length	Number of spikeletes	Awning
TGMS-1	UPRI-99-70-1	110	86.24*	11.5	Green	Intermediate	21.08	236.0*	Present
TGMS-2	UPRI-99-71-1	112*	72.30	13.2*	Green	Intermediate	24.00*	213.0*	Absent
TGMS-3	UPRI-99-71-2	117*	86.63*	9.7	Green	Open	23.78*	152.0	Absent
TGMS-4	UPRI-99-73-1	117*	82.66	10.2	Green	Intermediate	23.32*	239.0*	Absent
TGMS-5	UPRI-99-73-2	119*	84.58*	14.2*	Green	Intermediate	22.40	232.0*	Absent
TGMS-6	UPRI-99-73-3	114*	76.54	12.3*	Green	Intermediate	21.84	127.6	Absent
TGMS-7	UPRI-99-73-4	112*	76.31	8.9	Purple	Intermediate	21.48	183.0	Absent
TGMS-8	UPRI-99-74-3	108	80.35	7.5	Purple	Intermediate	23.80*	165.3	Present
TGMS-9	UPRI-99-79-1	112*	88.45*	11.8	Purple	Open	23.58*	296.3*	Absent
TGMS-10	UPRI-97-60-1	110	88.56*	12.4*	Purple	Open	23.46*	204.3*	Absent
TGMS-11	UPRI-99-72-1	114*	78.90	12.1*	Purple	Intermediate	24.10*	173.0	Absent
TGMS-12	UPRI-99-72-3	114*	80.36	13.0*	Purple	Intermediate	25.28*	156.6	Absent
TGMS-13	UPRI-99-72-4	112*	88.42*	14.2*	Purple	Intermediate	22.64	148.8	Absent
TGMS-14	UPRI-99-74-1	113*	88.64*	12.0	Purple	Intermediate	24.04*	145.3	Present
TGMS-15	UPRI-99-74-4	110	88.35*	15.1*	Purple	Intermediate	22.58	161.3	Present
TGMS-16	UPRI-99-75-1	110	84.41*	11.5	Purple	Open	21.92	175.3	Present
TGMS-17	UPRI-99-78-1	114*	88.50*	14.5*	Green	Open	23.48*	303.5*	Absent
TGMS-18	UPRI-97-60-8	096	88.34*	12.6*	Purple	Open	22.08	260.0*	Absent
Mean value		111.88	83.80	12.04			23.05	198.46	

*, significant at mean value

4.4.1.11 Glume angle

The glume angle varied from 18° (UPRI-99-74-4) to 33° (UPRI-99-73-1). Nine lines showing significantly high mean value. The lines which showing highest glume angle viz. UPRI-99-73-1 (33.0), UPRI-99-74-3 (28.4), UPRI-99-72-1 (28.2), UPRI-99-74-1 (27.2) and UPRI-99-73-4 (27.0). **Thiagarajan *et al.* (2010)** reported the glume angle range from 15° to 25°, **Singh and Rang (1999)** reported the range 35° to 48° and **Ravneet S. Behta *et al.* (2007)** reported the range of 23.43° to 30.20°.

4.4.1.12 Panicle exertion percentage

Panicle exertion per-cent varied from 69.73 % (UPRI-99-71-1) to 84.10 % (UPRI-99-71-2). The ten lines showing the significantly high mean value for panicle exertion percentage out of ten five lines with higher percentage of panicle exertion viz. UPRI-99-71-2 (84.10), UPRI-99-73-1 (82.82), UPRI-99-73-2 (82.43), UPRI-99-74-3 (81.97) and UPRI-97-60-8 (81.86).

4.4.1.13 Stigma exertion percentage

The nine lines were showing significantly high mean value. The exerted stigma range varied from 26.67 (UPRI-99-79-1) to 80.05 (UPRI-99-72-1). The lines were with highest percentage of exerted stigma viz. UPRI-99-72-1 (80.05), UPRI-99-78-1 (79.50), UPRI-99-73-1 (77.19), UPRI-99-74-3 (74.34) and UPRI-99-71-1 (73.96).

4.4.1.14 Anthesis time

Anthesis time varied from 8:05 (UPRI-99-74-3) to 10:15 (UPRI-97-60-8). The anthesis time starts from 8:00 and end to 12:30 for all the lines.

4.4.1.15 Anthesis duration in minutes

Anthesis time duration in minutes varied from 125 (UPRI-99-79-1) to 205 (UPRI-99-74-3). Anthesis time duration long is good for pollination and hybridization for production of hybrid. The best five lines are which have long duration for anthesis viz. UPRI-99-74-3 (205), UPRI-99-71-2 (185), UPRI-99-72-1 (180), UPRI-99-73-3 (170) and UPRI-99-75-1 (170).

Table 4.15: Floral and morphological traits recorded in TGMS lines

Line number	TGMS name	Apiculous pigmentation	Stigma color	Glume angle	Panicle exertion (%)	Stigma exertion (%)	Anthesis time	Anthesis duration (Min.)	Pollen sterility
TGMS-1	UPRI-99-70-1	Absent	Purple	22.5	81.35*	49.30	10:00	130	84.95
TGMS-2	UPRI-99-71-1	Absent	White	24.6	69.73	73.96*	8:50	165	79.07
TGMS-3	UPRI-99-71-2	Absent	Purple	26.0*	84.10*	55.10	9:00	185	87.67*
TGMS-4	UPRI-99-73-1	Absent	White	33.0*	82.82*	77.19*	9:30	135	98.84*
TGMS-5	UPRI-99-73-2	Absent	White	24.2	82.43*	64.31*	8:45	165	97.64*
TGMS-6	UPRI-99-73-3	Absent	Purple	26.2*	81.09*	40.59	8:55	170	90.18*
TGMS-7	UPRI-99-73-4	Purple	Purple	27.0*	78.58*	37.12	9:30	150	89.81*
TGMS-8	UPRI-99-74-3	Purple	Purple	28.4*	81.97*	74.34*	8:05	205	79.58
TGMS-9	UPRI-99-79-1	Purple	Purple	23.8	78.84*	26.67	9:50	125	96.75*
TGMS-10	UPRI-97-60-1	Purple	Purple	25.0*	74.14	38.09	9:40	160	92.38*
TGMS-11	UPRI-99-72-1	Purple	Purple	28.2*	69.76	80.05*	8:50	180	87.09*
TGMS-12	UPRI-99-72-3	Purple	Purple	25.2*	73.04	47.97	9:10	140	96.64*
TGMS-13	UPRI-99-72-4	Purple	Purple	24.2	72.92	72.51*	9:00	145	88.43*
TGMS-14	UPRI-99-74-1	Purple	Purple	27.2*	70.52	64.69*	9:05	165	88.22*
TGMS-15	UPRI-99-74-4	Purple	Purple	18.0	74.44	49.20	9:15	150	87.79*
TGMS-16	UPRI-99-75-1	Purple	Purple	21.0	81.28*	69.39*	8:45	170	83.19
TGMS-17	UPRI-99-78-1	Absent	Purple	20.6	77.06	79.50*	10:10	130	57.62
TGMS-18	UPRI-97-60-8	Purple	Purple	18.4	81.86*	26.80	10:15	135	44.32
Mean value				24.63	77.55	57.04			85.00

4.4.1.16 Pollen sterility

Pollen sterility (Fig.4.1) study showed that the mean of sterility was 85%. The range of pollen sterility percentage was 44.32 % (UPRI-97-60-8) to 98.84 % (UPRI-99-73-1). Therefore lines showed high value over mean. The lines are which showed maximum sterility in the field viz. UPRI-99-73-1 (98.84), UPRI-99-73-2 (97.64), UPRI-99-79-1 (96.75), UPRI-99-72-3 (96.64) and UPRI-97-60-1 (92.38).

For characterization of different TGMS lines based on floral and morphological traits, different researchers found same findings for different traits with high mean value viz. **Salgotra et al. (2012)** and **Thiyagarajan et al. (2010)**, studied the effective tiller number per plant, panicle exertion, number of spikeletes, glume angle, pollen sterility **Ramakrishna et al. (2006)** and **Singh et al. (2011)**, plant height, panicle type, panicle exertion, stigma exertion, Apiculous pigmentation, stigma color, time of anthesis, pollen sterility, duration of anthesis and glume angle used for characterization of different lines. Floral biology and morphological characterization of TGMS lines **Celine et al. (2014)** studied the stigma color, pollen sterility, apiculous color and panicle type. **Kavithamani et al. (2012)** studied the traits viz. stigma exertion, days to flowering, pollen sterility and stigma color.

Characterization of morphological traits is essential for distinguishing cultivars; grow out test, seed certification and for gene bank deposition. Therefore, characterization based on phenology for eighteen TGMS lines was carried out. A thorough understanding of the floral biology is essential in TGMS system as it switches over to fertility/sterility conditions based on temperature influence (**Ramakrishna et al. 2006**). **Saran et al. (1971)** reported that duration of floret opening was positively correlated with percentage of sterility. High frequency of exerted stigma facilitates stigma reception of naturally out crossed pollen grains and higher seed set. **Oka (1998)** stated that out crossing in rice depended on the capacity of stigma to receive alien pollen and the capacity of anthers to emit pollen to pollinate other plants in the proximity. Not a single line was found to be good for all the traits however different lines were good for different traits. The lines which showed high panicle exertion, high stigma exertion, more anthesis duration and high pollen sterility could be used successfully in two line hybrid seed production.

The eighteen TGMS line were evaluated for critical sterility and fertility temperature in three seasons. Five randomly selected panicles from each TGMS line were covered by butter paper bag at heading stage to insure selfing; this was intended for the measurement of the spikelet fertility due to selfing. The bagged panicles were harvested at maturity. The average of the spikelet fertility from the five bagged panicle from each TGMS line was computed. Correlation between spikeletes fertility and maximum and minimum daily temperature up to thirty days before heading were computed to determine the critical stage where temperature influences the sterility/fertility expression of the TGMS lines.

Temperature data were obtained from the agrometrology department of G.B. Pant University of Agriculture & Technology, Pantnagar. A maximum and minimum daily temperature up to thirty days before heading was obtained. This period corresponds to the panicle development of rice plant from the differentiation of the first bract primordium to the time the spikelets completely formed (Virmani and Sharma 1993).

Table 4.15 Details of average temperature between panicle initiations to flowering (Kharif, 2013)

Lines	Name	Date of 50% flowering	Average temperature between panicle initiation to flowering			seed setting
			Max.	Min.	Average	
TGMS-1	UPRI-99-70-1	05/09/2013	32.39	25.21	29.51	35.0
TGMS-2	UPRI-99-71-1	04/09/2013	32.43	25.33	28.88	0.0
TGMS-3	UPRI-99-71-2	02/09/2013	32.45	25.39	28.92	0.0
TGMS-4	UPRI-99-73-1	11/09/2013	32.29	24.85	28.57	0.0
TGMS-5	UPRI-99-73-2	11/09/2013	32.29	24.85	28.57	0.0
TGMS-6	UPRI-99-73-3	13/09/2013	32.31	24.75	28.53	4.0
TGMS-7	UPRI-99-73-4	11/09/2013	32.29	24.85	28.57	0.0
TGMS-8	UPRI-99-74-3	07/09/2013	32.32	25.11	28.71	0.0
TGMS-9	UPRI-99-79-1	09/09/2013	32.28	25.02	28.65	14.0
TGMS-10	UPRI-97-60-1	09/09/2013	32.28	25.02	28.65	0.0
TGMS-11	UPRI-99-72-1	08/09/2013	32.31	25.07	28.69	0.0
TGMS-12	UPRI-99-72-3	10/09/2013	32.25	24.93	28.59	2.0
TGMS-13	UPRI-99-72-4	09/09/2013	32.28	25.02	28.65	5.0
TGMS-14	UPRI-99-74-1	11/09/2013	32.29	24.85	28.57	0.0
TGMS-15	UPRI-99-74-3	07/09/2013	32.31	25.11	28.71	0.0
TGMS-16	UPRI-99-75-1	09/09/2013	32.28	25.02	28.65	2.0
TGMS-17	UPRI-99-78-1	13/09/2013	32.31	24.75	28.53	1.0
TGMS-18	UPRI-97-60-8	09/09/2013	32.28	25.02	28.65	9.0

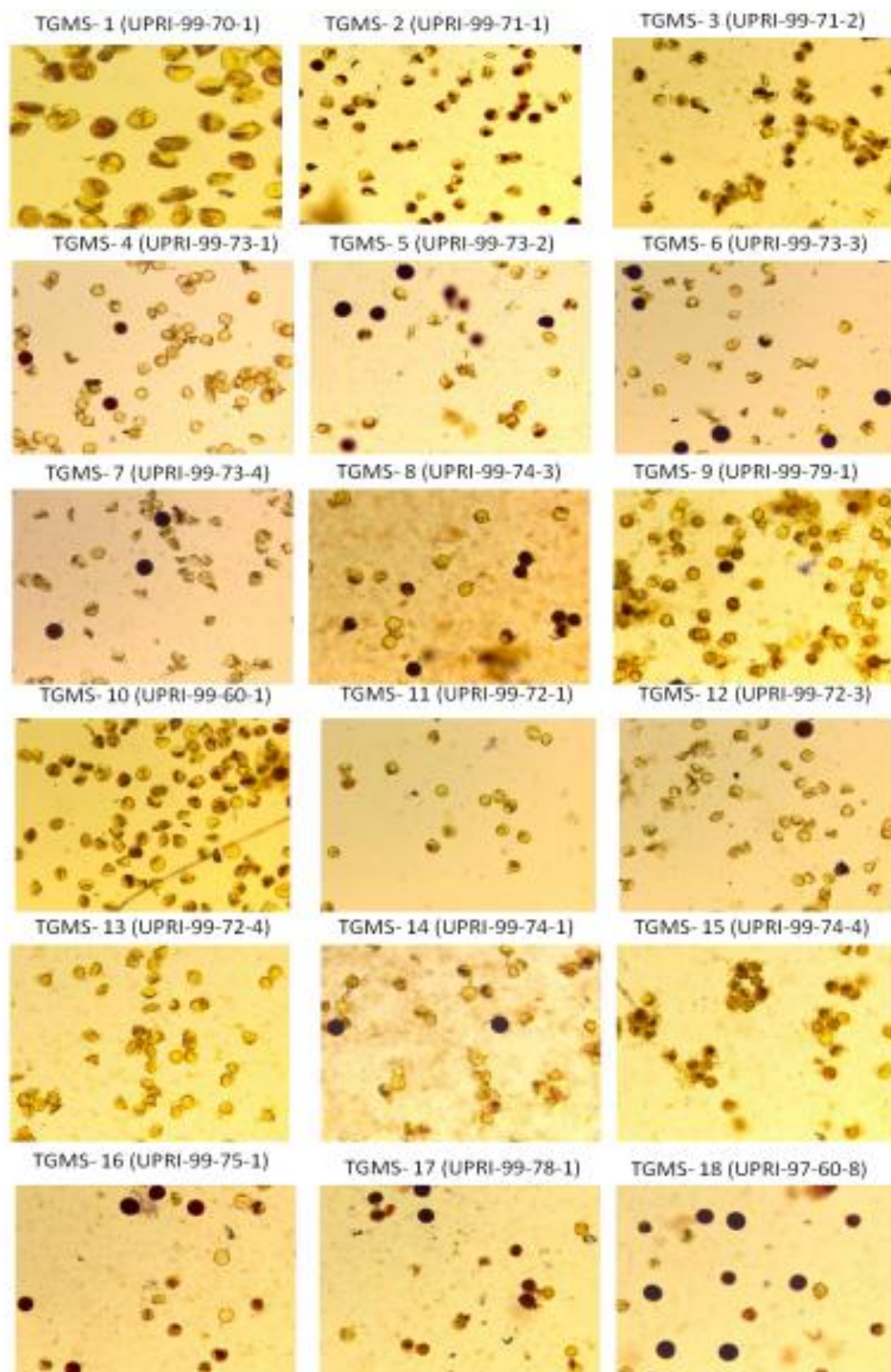


Fig. 4.1 Microscopic pollen sterility study of different TGMS lines of rice

**Table 4.16 Details of average temperature between panicle initiations to flowering
(Offseason, 2014)**

Lines	Name	Date of 50% flowering	Average temperature between panicle initiation to flowering			seed setting
			Max.	Min.	Average	
TGMS-1	UPRI-99-70-1	18/06/2014	38.74	24.83	31.78	0.0
TGMS-2	UPRI-99-71-1	20/06/2014	38.45	25.12	31.78	0.0
TGMS-3	UPRI-99-71-2	23/06/2014	38.17	25.51	31.84	0.0
TGMS-4	UPRI-99-73-1	06/07/2014	36.95	26.13	31.54	0.0
TGMS-5	UPRI-99-73-2	20/07/2014	33.21	25.98	29.59	0.0
TGMS-6	UPRI-99-73-3	30/06/2014	37.84	26.05	31.94	0.0
TGMS-7	UPRI-99-73-4	02/07/2014	37.37	26.12	31.74	0.0
TGMS-8	UPRI-99-74-3	23/06/2014	38.17	25.51	31.84	0.0
TGMS-9	UPRI-99-79-1	02/07/2014	37.37	26.12	31.74	0.0
TGMS-10	UPRI-97-60-1	21/06/2014	38.36	25.30	31.83	0.0
TGMS-11	UPRI-99-72-1	23/06/2014	38.17	25.51	31.84	0.0
TGMS-12	UPRI-99-72-3	28/06/2014	37.80	25.94	31.87	0.0
TGMS-13	UPRI-99-72-4	02/07/2014	37.37	26.12	31.74	0.0
TGMS-14	UPRI-99-74-1	23/06/2014	38.17	25.51	31.84	0.0
TGMS-15	UPRI-99-74-3	23/06/2014	38.17	25.51	31.84	0.0
TGMS-16	UPRI-99-75-1	19/06/2014	38.64	25.01	31.82	0.0
TGMS-17	UPRI-99-78-1	14/06/2014	38.69	24.03	31.36	0.0
TGMS-18	UPRI-97-60-8	19/06/2014	38.64	25.01	31.82	0.0

**Table 4.17 Details of average temperature between panicle initiations to flowering
(Kharif, 2014)**

Lines	Name	Date of 50% flowering	Average temperature between panicle initiation to flowering			seed setting main lines
			Max.	Min.	Average	
TGMS-1	UPRI-99-70-1	08/09/2014	33.33	25.69	29.51	10.0
TGMS-2	UPRI-99-71-1	10/09/2014	33.43	25.63	29.53	0.0
TGMS-3	UPRI-99-71-2	15/09/2014	33.12	25.20	29.16	0.0
TGMS-4	UPRI-99-73-1	15/09/2014	33.12	25.20	29.16	0.0
TGMS-5	UPRI-99-73-2	17/09/2014	33.27	25.13	29.20	0.0
TGMS-6	UPRI-99-73-3	12/09/2014	33.28	25.50	29.39	3.0
TGMS-7	UPRI-99-73-4	10/09/2014	33.43	25.63	29.53	0.0
TGMS-8	UPRI-99-74-3	06/09/2014	33.40	25.82	29.61	0.0
TGMS-9	UPRI-99-79-1	10/09/2014	33.43	25.63	29.53	11.0
TGMS-10	UPRI-97-60-1	08/09/2014	33.33	25.69	29.51	0.0
TGMS-11	UPRI-99-72-1	12/09/2014	33.28	25.50	29.39	0.0
TGMS-12	UPRI-99-72-3	12/09/2014	33.28	25.50	29.39	0.0
TGMS-13	UPRI-99-72-4	10/09/2014	33.43	25.63	29.53	0.0
TGMS-14	UPRI-99-74-1	11/09/2014	33.34	25.55	29.44	0.0
TGMS-15	UPRI-99-74-3	08/09/2014	33.33	25.69	29.51	0.0
TGMS-16	UPRI-99-75-1	08/09/2014	33.33	25.69	29.51	0.0
TGMS-17	UPRI-99-78-1	12/09/2014	33.28	25.50	29.39	0.0
TGMS-18	UPRI-97-60-8	04/09/2014	33.31	25.83	29.57	3.0

Table 4.18 Critical sterility temperature for different TGMS lines

S. No.	TGMS Lines	Critical temperature for sterility (°C)	seed setting		
			2013	2014	
			Main season	Main season	off-season
1.	TGMS-1	>29.51	35.0	10.0	0.0
2.	TGMS-2	28.88	0.0	0.0	0.0
3.	TGMS-3	28.92	0.0	0.0	0.0
4.	TGMS-4	28.57	0.0	0.0	0.0
5.	TGMS-5	28.57	0.0	0.0	0.0
6.	TGMS-6	>28.53	4.0	3.0	0.0
7.	TGMS-7	28.57	0.0	0.0	0.0
8.	TGMS-8	28.71	0.0	0.0	0.0
9.	TGMS-9	>28.65	14.0	11.0	0.0
10.	TGMS-10	28.65	0.0	0.0	0.0
11.	TGMS-11	28.69	0.0	0.0	0.0
12.	TGMS-12	28.59	2.0	0.0	0.0
13.	TGMS-13	29.53	5.0	0.0	0.0
14.	TGMS-14	28.57	0.0	0.0	0.0
15.	TGMS-15	28.71	0.0	0.0	0.0
16.	TGMS-16	29.51	2.0	0.0	0.0
17.	TGMS-17	29.39	1.0	0.0	0.0
18.	TGMS-18	>28.65	9.0	3.0	0.0

The minimum critical temperature for sterility was observed in TGMS-17 (28.53°C) and maximum for TGMS-1 (29.51°C) (Table-4.18). Among eighteen TGMS lines fourteen lines were found completely sterile in *kharif* 2013 and 2014 and in offseason of 2014. In 2014 offseason all the lines showed complete sterility because average temperature was high 29.59-31.94°C. The details of TGMS lines in this reference are given below:

- 1) **TGMS-1 (UPRI-99-70-1)** the line showed spikelet fertility during main *kharif* 2013 and 2014 season. Average seed setting was found be 35 and 10 respectively meaning that for this the line requirement of critical temperature is more than 29.51 in *kharif*. During offseason temperature was high (31.78°C) thus no seed setting was found.
- 2) **TGMS-2 (UPRI-99-71-1)** the line was found completely sterile during all the three seasons. So critical sterility temperature determined as 28.88°C.
- 3) **TGMS-3 (UPRI-99-71-2)** in this line no seed setting was in three seasons. The critical sterility temperature was 28.92°C.
- 4) **TGMS-4 (UPRI-99-73-1)** the line was found completely sterile during all the three seasons at 28.57°C temperature. So this was the critical sterility temperature for this line.

- 5) **TGMS-5 (UPRI-99-73-2)** the line was found completely sterile during all the three seasons. So critical sterility temperature determined as 28.57°C.
- 6) **TGMS-6 (UPRI-99-73-3)** the line showed spikelet fertility during main *kharif* 2013 and 2014 season. Average seed setting was found 4 and 3 respectively meaning that for this line requirement of critical temperature is more than 28.53°C in *kharif*. During offseason temperature was high (31.94°C) thus no seed setting was found.
- 7) **TGMS-7 (UPRI-99-73-4)** the line was found completely sterile during all the three seasons at 28.57°C temperature. So this was the critical sterility temperature for this line.
- 8) **TGMS-8 (UPRI-99-74-3)** the line was found completely sterile during all the three seasons at 28.71°C temperature. So this was the critical sterility temperature for this line.
- 9) **TGMS-9 (UPRI-99-79-1)** the line showed spikelet fertility during main *kharif* 2013 and 2014; average seed setting was found 14 and 11 respectively it means for this line requirement of critical temperature more than 28.65°C in *kharif*. During offseason temperature was high (31.74°C) thus no seed setting was found.
- 10) **TGMS-10 (UPRI-97-60-1)** the line was found completely sterile during all the three seasons at 28.65°C temperature. So this was the critical sterility temperature for this line.
- 11) **TGMS-11 (UPRI-99-72-1)** the line was found completely sterile during all the three seasons at 28.69°C temperature. So this was the critical sterility temperature for this line.
- 12) **TGMS-12 (UPRI-99-72-3)** in 2013 *kharif* in this line found two fertile spikelet and other two seasons, *kharif* and offseason in 2014 found complete sterility. The average temperature in *kharif*, 2013 was 28.59°C and *kharif* 2014 average temperature was 29.39°C it means this was critical sterility temperature for this line.
- 13) **TGMS-13 (UPRI-99-72-4)** in 2013 *kharif* in this line found five fertile spikelet and other two seasons, *kharif* and offseason in 2014 found complete sterility. The average temperature in *kharif*, 2013 was 28.65°C and *kharif* 2014 average

temperature was 29.53°C it means this was critical sterility temperature for this line.

14) TGMS-14 (UPRI-99-74-1) the line was found completely sterile during all the three seasons at 28.57°C temperature. So this was the critical sterility temperature for this line.

15) TGMS-15 (UPRI-99-74-3) the line was found completely sterile during all the three seasons at 28.71°C temperature. So this was the critical sterility temperature for this line.

16) TGMS-16 (UPRI-99-75-1) in 2013 *kharif* in this line found two fertile spikelet and other two seasons, *kharif* and offseason in 2014 found complete sterility. The average temperature in *kharif*, 2013 was 28.65°C meaning that this was not critical sterility temperature. In *kharif*, 2014 average temperature was 29.51°C and found complete sterility, so this was the critical sterility temperature for this line.

17) TGMS-17 (UPRI-99-78-1) in 2013 *kharif* in this line found one fertile spikelet and other two seasons, *kharif* and offseason in 2014 found complete sterility. The average temperature in *kharif*, 2013 was 28.53°C meaning that this was not critical sterility temperature. In *kharif*, 2014 average temperature was 29.39°C and found complete sterility, so this was the critical sterility temperature for this line.

18) TGMS-18 (UPRI-97-60-8) the line showed spikelet fertility during main *kharif* 2013 and 2014. Average seed setting were found 9 and 3 respectively meaning that for this line requirement of critical temperature is more than 28.65°C in *kharif*. During offseason temperature was high (31.82°C) thus no seed setting was found.

The critical fertility temperature for all the lines was less than 28°C and below this temperature all the lines showed fertility.

The different researchers have reported the average and critical temperature for different TGMS lines **Lohithaswa H.C. et al., (2000)** found 35/23°C and **Viraktamath & Virmani (2001)** found 32/24°C temperature for sterility. **Sanchez and Virmani (2005)** did correlation analyses between spikelet fertility and maximum and daily mean temperatures up to days before heading to determine the critical stage where temperature influences the sterility/fertility expression of the TGMS lines and found that 32°C

temperature for sterility. **Ramkrishna S. et al., (2006)** and **Latha and Thiyagarajan (2010)** found $>30^{\circ}\text{C}$ temperature for sterility. **Salgotra et al. (2012)** and **Celine et al., (2014)** found 35.4°C critical temperature for sterility..

Therefore among the eighteen TGMS lines ten lines viz, TGMS-2, TGMS-3, TGMS-4, TGMS-5, TGMS-7, TGMS-8, TGMS-10, TGMS-11, TGMS-14 and TGMS-15 showed complete sterility at critical temperature 28.53 to 28.92°C . These can be used in two line hybrid development.

Out of eighteen lines four lines viz. TGMS-1, TGMS-6, TGMS-9 and TGMS-18 showed fertile spikelets in both main season during 2013 and 2014 but showed complete sterile spikelet in 2014 offseason. It means these lines required high temperature compare to main season because in offseason the average temperatures for these lines were 31.74 to 31.94°C , so these lines required $>29^{\circ}\text{C}$ critical temperature for complete sterility.

Another four lines viz. TGMS-12, TGMS-13, TGMS-16 and TGMS-17 found that seed setting in *kharif*, 2013 and complete sterility in *kharif* and offseason 2014. It means the critical sterility temperature for these lines slightly higher 29.39 - 29.53°C than the 2013 *kharif* season.

Therefore, in Pantnagar situation to get complete sterility these lines should be sown in the last week of May and transplanting be done after 25 to 30 days of seedlings for two line hybrid programme would be appropriate.

4.4.2 Molecular characterization of TGMS lines

The 18 TGMS lines of rice namely; UPRI-99-70-1, UPRI99-71-1, UPRI-99-71-2, UPRI-99-73-1, UPRI-99-73-2, UPRI-99-73-3, UPRI-99-73-4, UPRI-99-74-3, UPRI-99-79-1, UPRI-97-60-1, UPRI-99-72-1, UPRI-99-72-3, UPRI-99-72-4, UPRI-99-74-1, UPRI-99-74-3, UPRI-99-75-1, UPRI-99-78-1 and UPRI-97-60-8 were taken for diversity analysis using 26 simple sequence repeats (SSRs) primers. The overall results of SSRs analysis have been presented in Table-4.19. All sixteen primers gave clear and consistent amplification and amplified products were ultimately used for DNA profiling. The primer wise analysis of SSRs markers has been described on ensuing pages along with PCR amplification profiles (Fig.4.2 to 4.17).

Table 4.19: Range of SSR loci scored, number and size of exclusive loci amplified in the Rice genotypes

Sl. No.	Primer code	No. of alleles amplified	No. of polymorphic alleles	Percentage polymorphism	Range of amplified alleles (bp)	Exclusive alleles		Size of unique alleles (bp)	Major allele Frequency	Gene Diversity	PIC Value
						Number	Name of genotype				
1	RM154	4	4	100	180-210bp	1	TGMS-1	180bp	0.8056	0.2809	0.2314
2	RM190	3	3	100	110-140bp	2	TGMS-3 & TGMS-4	120bp	0.7593	0.3395	0.2754
3	RM276	3	3	100	110-190bp	2	TGMS-10 & TGMS-17	150bp	0.8519	0.2510	0.2188
4	RM336	2	2	100	180-190bp	-	-	-	0.6389	0.4599	0.3540
5	RM287	4	4	100	120-310bp	2	TGMS-6	210 & 310bp	0.8611	0.2253	0.1926
6	RM279	4	4	100	180-200bp	2	TGMS-1 & TGMS-18	180 & 200bp	0.7500	0.2963	0.2340
7	RM327	2	2	100	205-210bp	2	TGMS-16 & TGMS-18	205bp	0.8889	0.1975	0.1780
8	RM324	3	3	100	190-200bp	2	TGMS-6 & TGMS-9	200bp	0.6852	0.3889	0.3040
9	RM341	3	3	100	140-180bp	-	-	-	0.6667	0.4074	0.3199
10	RM166	3	3	100	290-305bp	-	-	-	0.8333	0.2654	0.2255
11	RM418	2	2	100	295-300bp	2	TGMS-5 & TGMS-7	300bp	0.8889	0.1975	0.1780
12	RM335	5	5	100	100-180bp	1	TGMS-15	180bp	0.8111	0.2827	0.2358
13	RM499	1	0	0	100bp	-	-	-	0.5000	0.5000	0.3750
14	RM424	2	2	100	295-300bp	2	TGMS-5 & TGMS-7	300bp	0.8889	0.1975	0.1780
15	RM592	3	3	100	300-380bp	-	-	-	0.7037	0.3951	0.3145
16	RM204	3	3	100	110-200bp	1	TGMS-3	180bp	0.7778	0.3169	0.2552
Total		47	46								

RM154

The primer RM 154 pair revealed four alleles varied in size from 180 to 210bp (Fig.4.2). One unique band at 180bp was observed by these primers in TGMS-1 line

RM190

The marker RM190 amplified three alleles (Fig.4.3). The alleles amplified by marker were 100 percent polymorphic and varied in size from 110 to 150bp. One unique band at 120bp was observed in TGMS-3 and TGMS-4 lines.

RM276

Four alleles were amplified by the marker RM276 (Fig.4.4). This marker amplifies 100 percent polymorphism where size of alleles were found vary from 110 to 190bp. One unique band at 150bp was unique to TGMS-10 and TGMS-17

RM336

The marker RM336 was able to reveal only two alleles (Fig.4.5). The two loci amplified by this marker were 100 percent polymorphic. The amplified products were in the range of 180 to 190bp. No unique allele was observed by these primers in any of the lines.

RM287

The marker RM287 amplified four alleles (Fig.4.6), which were polymorphic. The size of alleles were in the range of 120 to 310bp. Two alleles at 210 and 310bp were unique to TGMS-6 line.

RM279

Four amplified alleles were revealed by this marker ((Fig.4.7). The size of alleles was noted to vary from 180 to 200bp. Two bands at 180 and 200bp were unique to TGMS-1 and TGMS-18 lines.

RM327

The marker RM327 revealed two polymorphic alleles (Fig.4.8). The size of alleles were vary from 205 to 210bp. One band at 205bp was unique to TGMS-16 and TGMS-18 lines.

RM324

Three alleles amplified by marker RM324 were found to be polymorphic (Fig.4.9). The amplified products were in the range of 190 to 200bp. One band at 200bp was unique to TGMS-6 and TGMS-9 lines.

RM341

The marker RM341 identified three polymorphic alleles in the range of 140 to 180bp (Fig.4.10). No unique allele was observed in any line.

RM166

Three polymorphic alleles amplified using marker RM166 were varied in size from 290to 305bp (Fig. 4.11). No unique allele was observed in any line.

RM418

The marker RM418 revealed two polymorphic alleles on Agarose gel (Fig.4.12). The amplified products were in the range of 295-300bp. One allele at 300bp was unique to TGMS-5 and TGMS-7 lines.

RM335

The marker RM335 identified five polymorphic alleles (Fig.4.13). The amplified products varied in size from 100 to 180bp. One allele at 180bp was unique to TGMS-15.

RM499

The RM499 was the marker which amplified only one allele of size 100bp (Fig.4.14).

RM424

The marker RM424 revealed two amplified alleles which were 100 percent polymorphic (Fig.4.15). The size of alleles varied from 295-300bp. One band at 300bp was unique to TGMS-5 and TGMS-7 lines.

RM592

Three polymorphic alleles were amplified using marker RM592 (Fig.4.16). The amplified products were in the range of 300 to 380bp. No unique locus was observed by this marker in any of the lines.

RM204

The marker RM204 revealed three amplified alleles and all the alleles were polymorphic (Fig.4.17). The amplification size was found to vary from 110 to 200bp. One band at 180bp was unique to TGMS-3.

4.4.3 Relationship among eighteen TGMS lines of Rice using SSRs markers

The SSR loci, with a scoring of allelic variation, illustrated the possibility to exploit the whole genotype information. The genotype information was based on allelic information *i.e.* whether the SSR band was present or absent in the PCR amplified product which was visualized in agarose gel by gel documentation system and bands were scored by using binary codes 0 (absence) and 1 (presence). The allelic frequency was also calculated by dominant scoring of the allele. The SSR data has been used to construct a dendrogram, as well as to identify genotypes and compute genetic distances. However, it is believed that the number of alleles might be more suitable than locus number to construct dendrograms. This is because different pairs of primers or loci have a different genetic diversity, which results in different allelic richness in the same group of accessions.

Jaccard's similarity coefficients among 18 TGMS lines of rice namely; UPRI-99-70-1 (1), UPRI99-71-1 (2), UPRI-99-71-2 (3), UPRI-99-73-1 (4), UPRI-99-73-2 (5),

RM-154

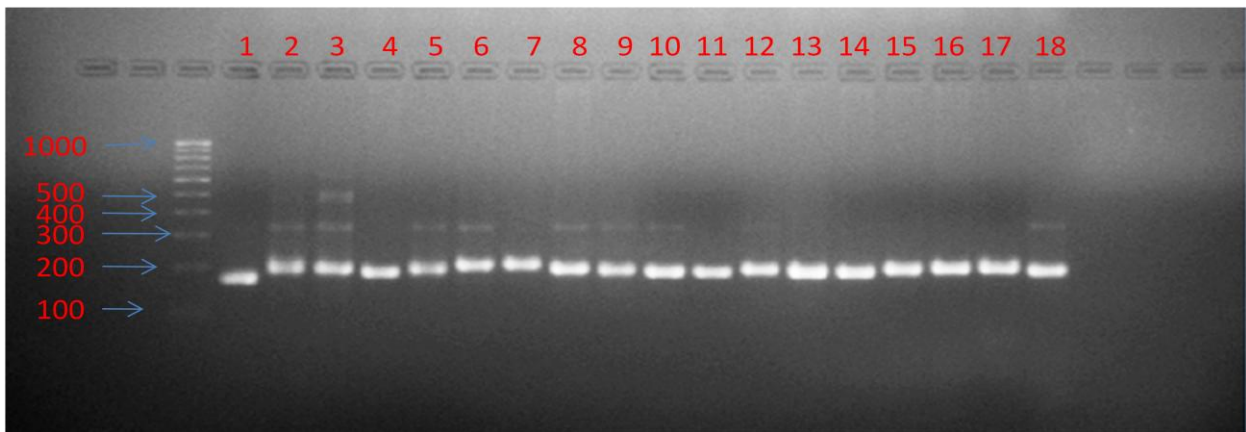


Fig. 4.2 SSR profile of eighteen rice TGMS lines with RM154 marker

RM-190

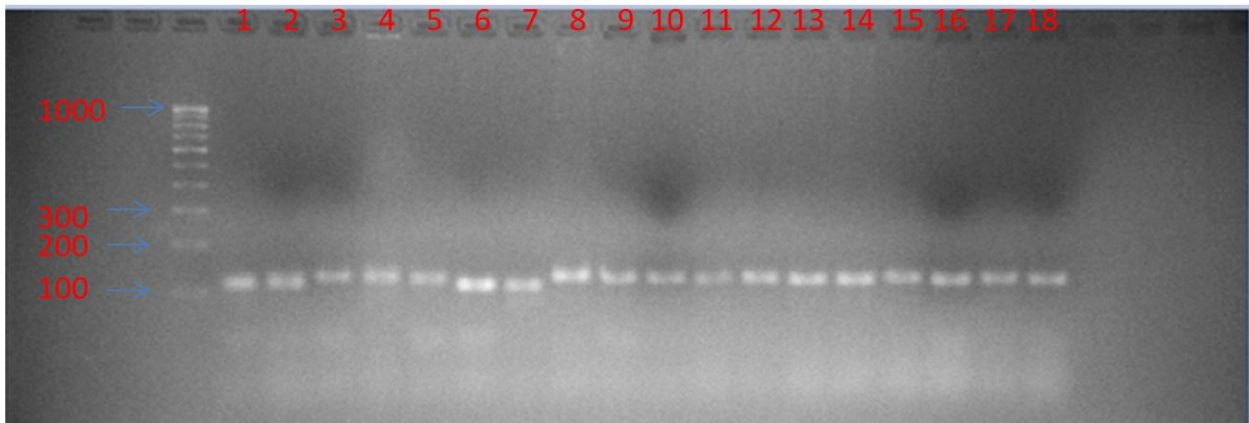


Fig. 4.3 SSR profile of eighteen rice TGMS lines with RM190 marker

RM-276

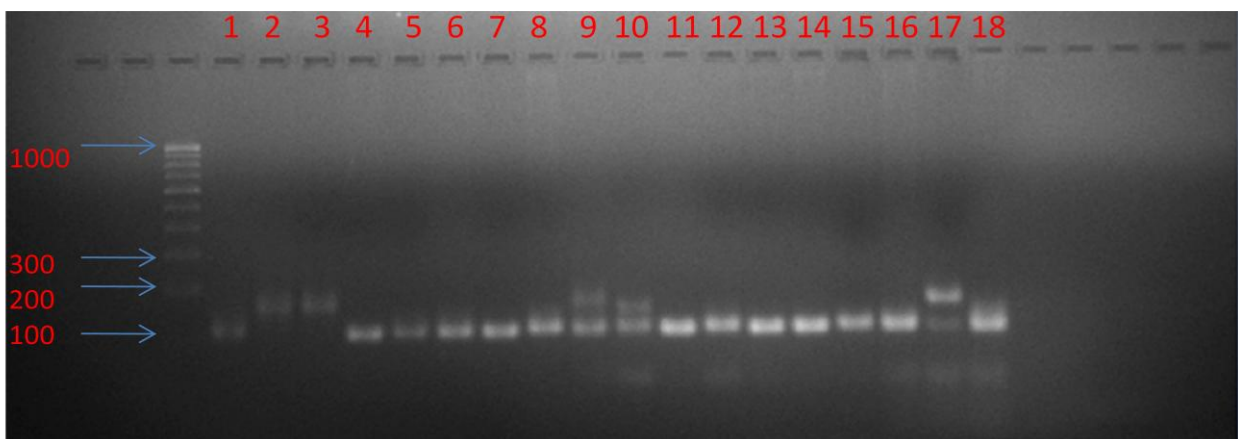


Fig. 4.4 SSR profile of eighteen rice TGMS lines with RM276 marker

RM-336

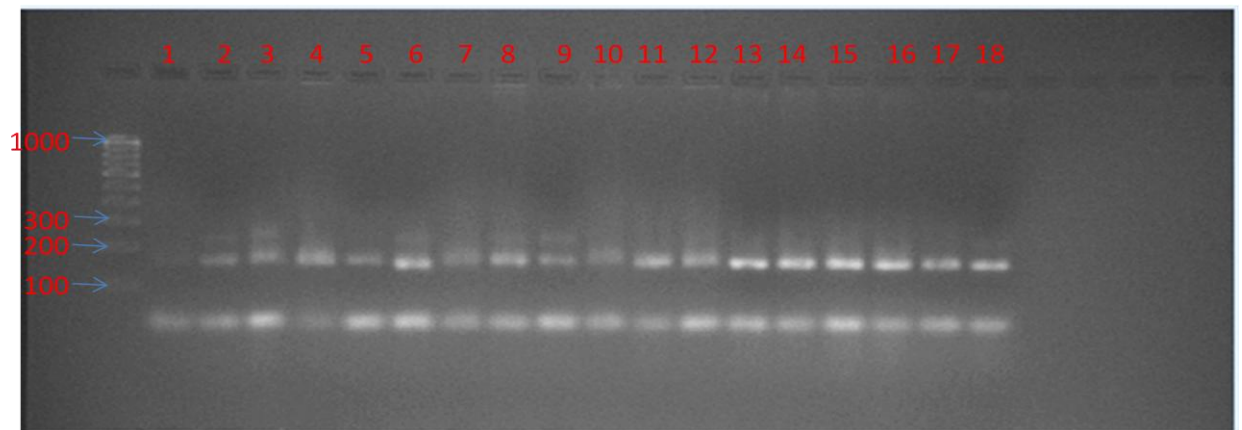


Fig. 4.5 SSR profile of eighteen rice TGMS lines with RM336 marker

RM-287

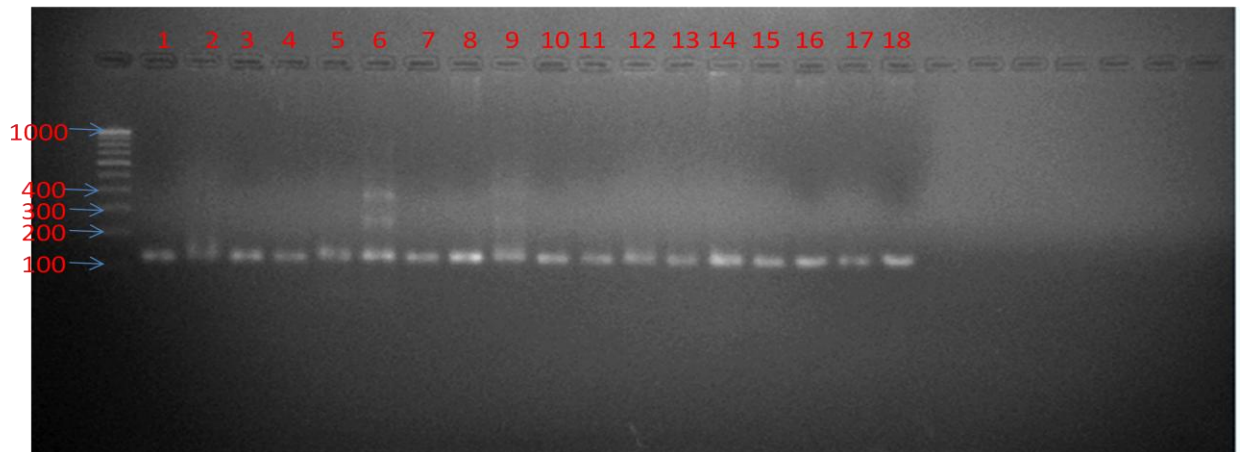


Fig. 4.6 SSR profile of eighteen rice TGMS lines with RM287 marker

RM-279

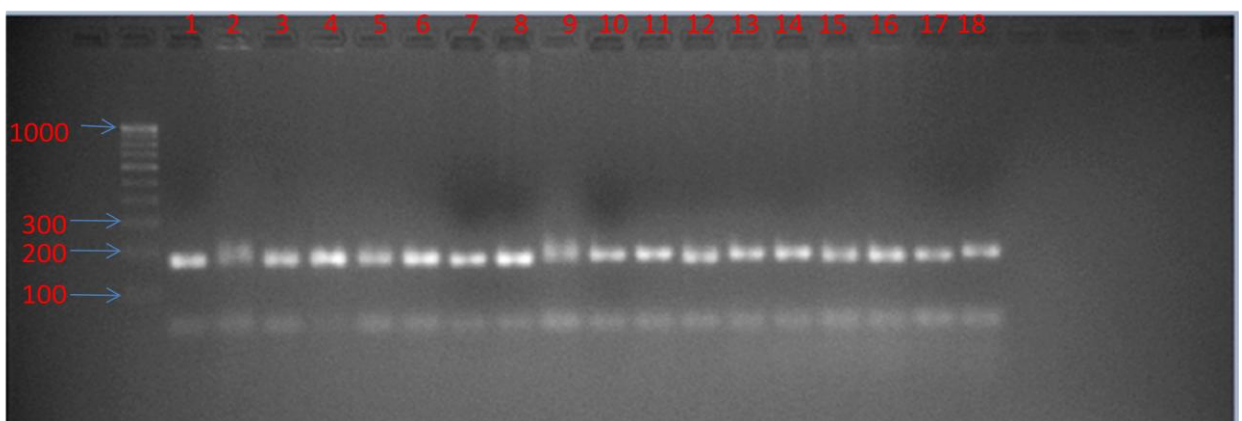


Fig. 4.7 SSR profile of eighteen rice TGMS lines with RM279 marker

RM-327

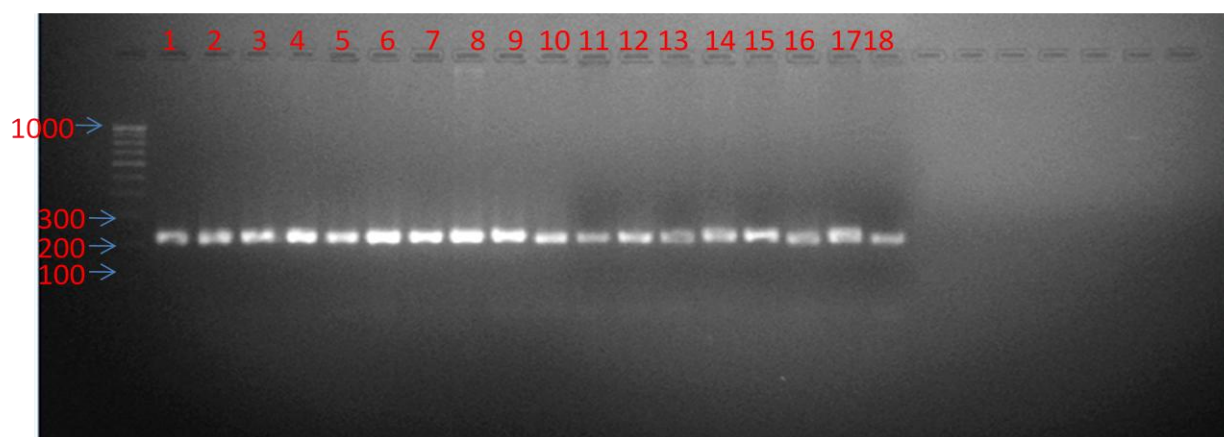


Fig. 4.8 SSR profile of eighteen rice TGMS lines with RM327 marker

RM-324

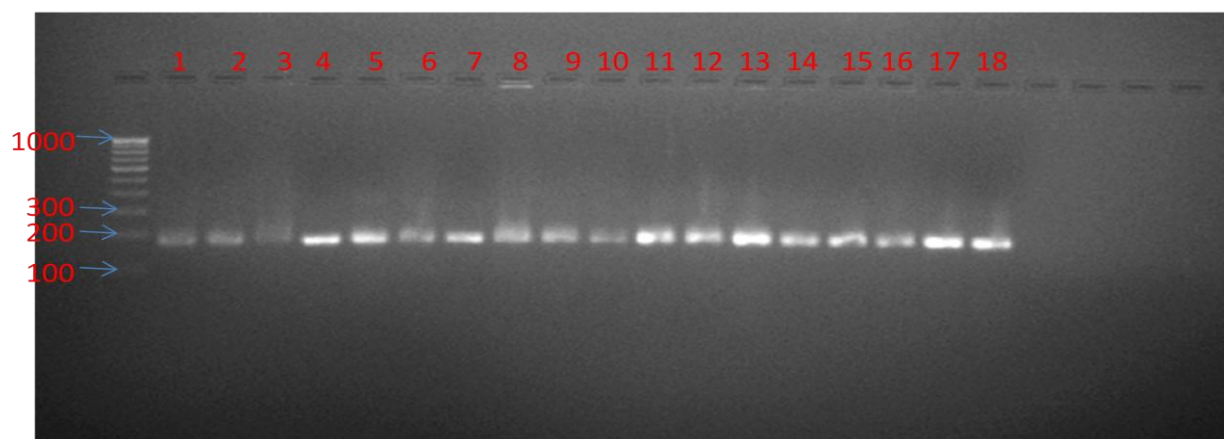


Fig. 4.9 SSR profile of eighteen rice TGMS lines with RM324 marker

RM-341

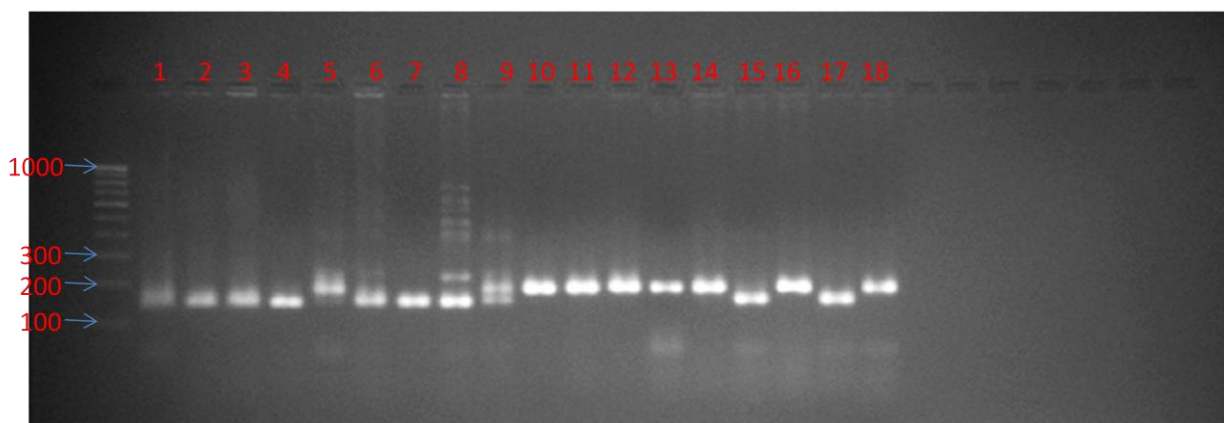


Fig. 4.10 SSR profile of eighteen rice TGMS lines with RM341 marker

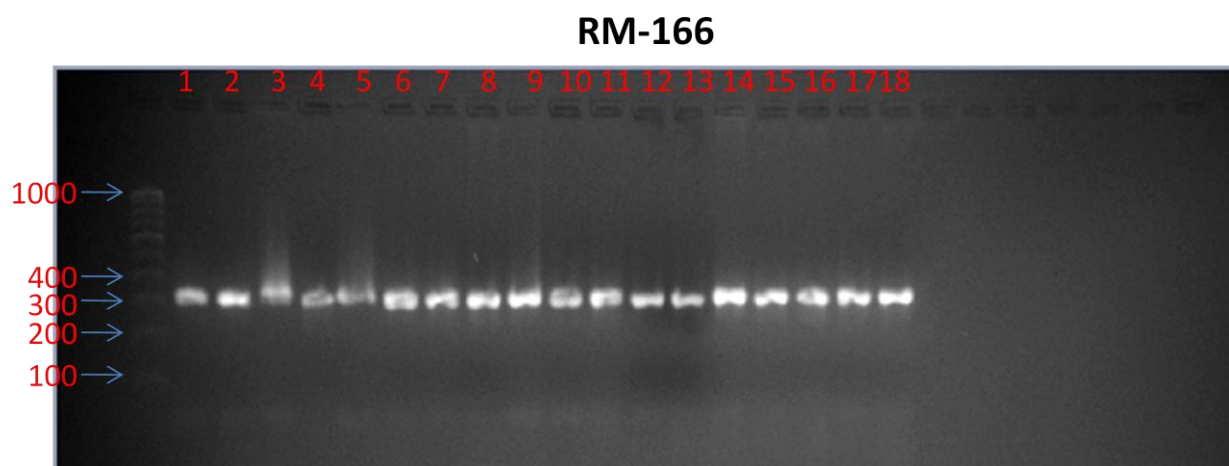


Fig. 4.11 SSR profile of eighteen rice TGMS lines with RM166 marker

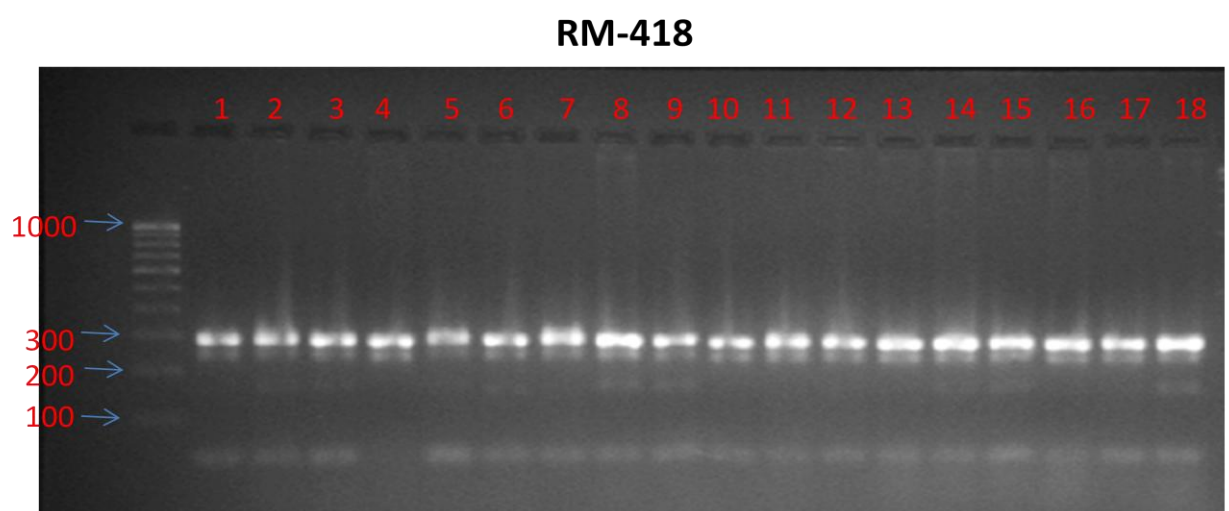


Fig. 4.12 SSR profile of eighteen rice TGMS lines with RM418 marker

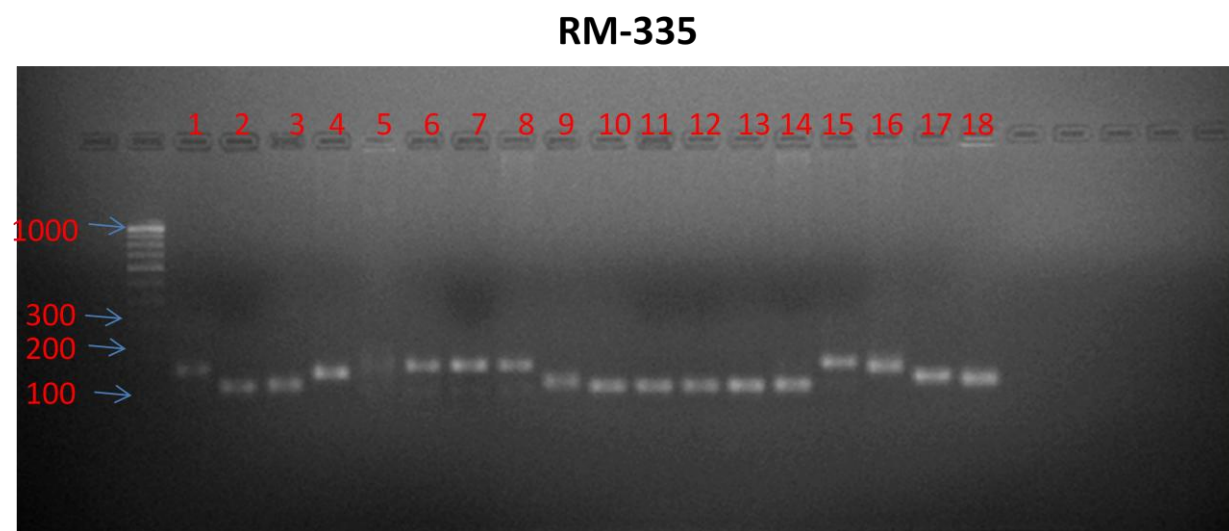


Fig. 4.13 SSR profile of eighteen rice TGMS lines with RM335 marker

RM-499

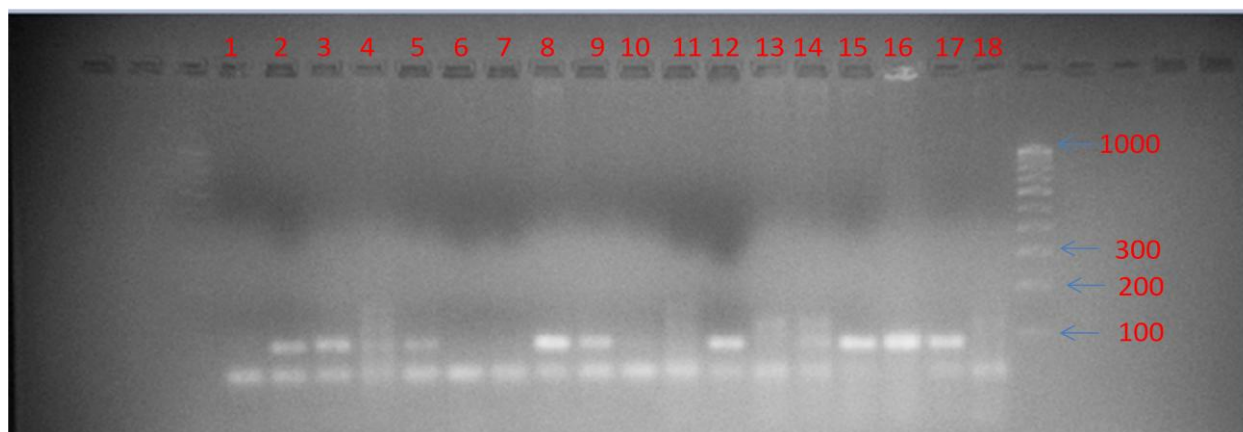


Fig. 4.14 SSR profile of eighteen rice TGMS lines with RM499 marker

RM-424

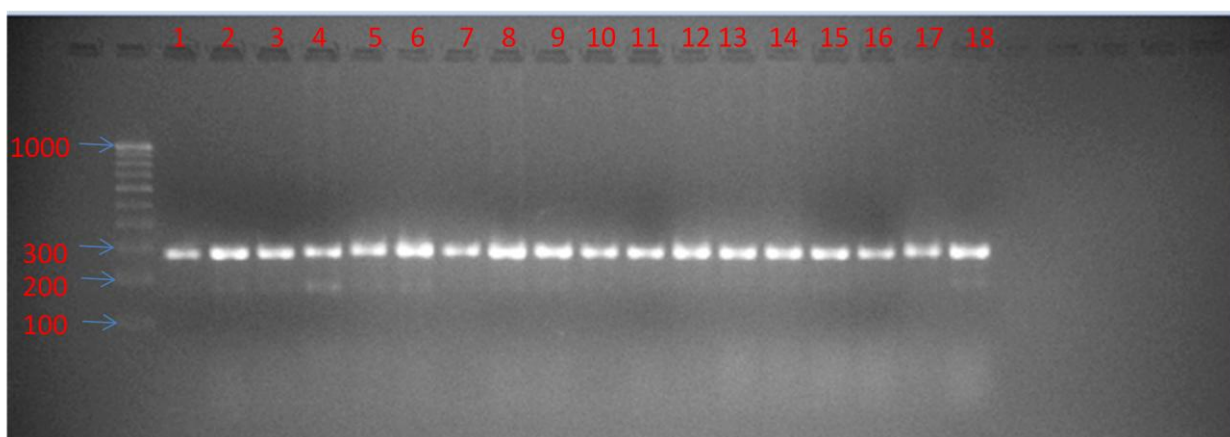


Fig. 4.15 SSR profile of eighteen rice TGMS lines with RM424 marker

RM-592

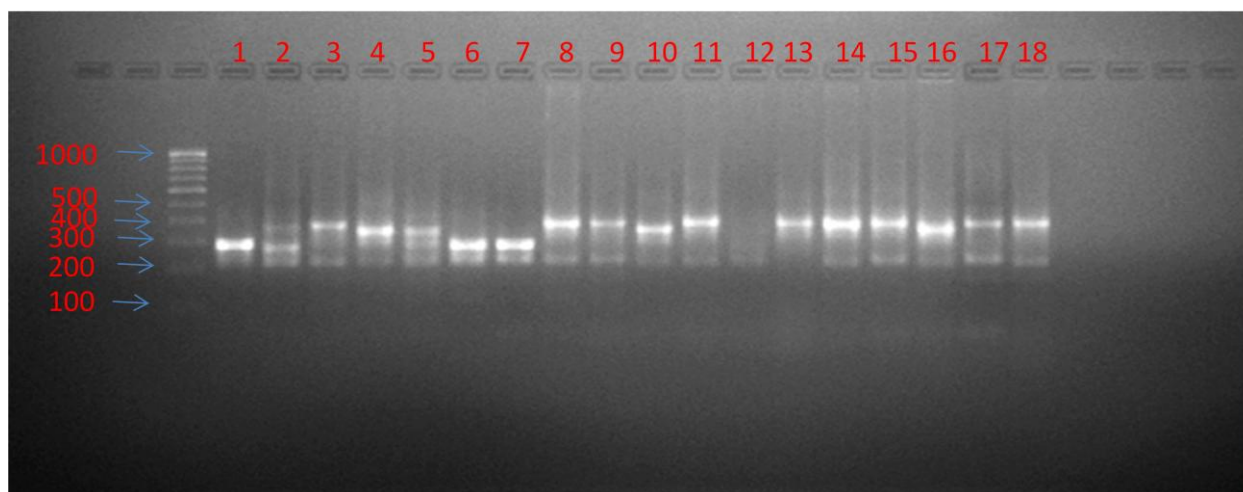


Fig. 4.16 SSR profile of eighteen rice TGMS lines with RM592 marker

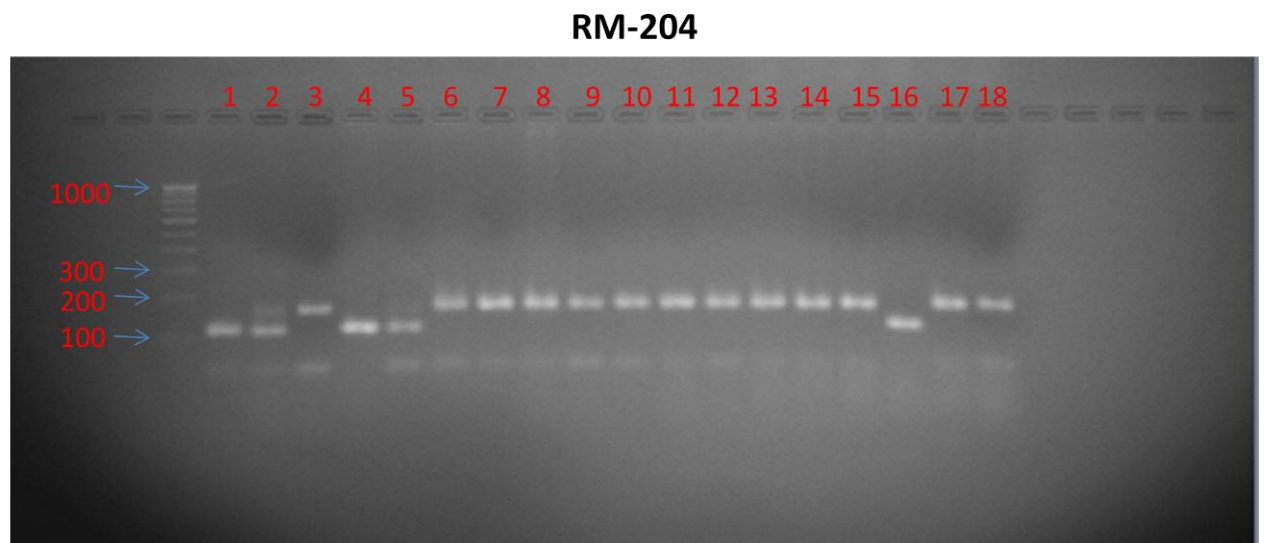


Fig. 4.17 SSR profile of eighteen rice TGMS lines with RM204 marker

UPRI-99-73-3 (6), UPR-99-73-4 (7), UPR-99-74-3 (8), UPR-99-79-1 (9), UPR-97-60-1 (10), UPR-99-72-1 (11), UPR-99-72-3 (12), UPR-99-72-4 (13), UPR-99-74-1 (14), UPR-99-74-3 (15), UPR-99-75-1 (16), UPR-99-78-1 (17) and UPR-97-60-8 (18) are presented in Table 4.20. Jaccard's similarity between the pair of genotype varied from maximum 0.97 between UPR-99-72-1 (11) and UPR-99-72-3 (12) to a minimum 0.46 between UPR-99-73-4 (7) and UPR-99-75-1 (16). Among the eighteen lines the three pairs with lowest genetic similarity (GS) value i.e., maximum diverse pairs were UPR-99-73-4 (7) and UPR-99-75-1 (16) (46% genetic similarity), UPR-99-73-3 (6) and UPR-99-71-2 (3) and UPR-97-60-8 (18) & UPR-99-73-2 (5) (with GS value 48%). Similarly, three pairs with maximum GS value i.e., minimum diversity in the experimental material of the present study were UPR-99-72-1 (11) and UPR-99-72-3 (12) (GS value 97%), UPR-99-72-4 (13) and UPR-99-72-1 (11) (GS value 95%) and between UPR-99-72-4 (13) and UPR-99-72-3 (12) (GS value 93%).

4.4.4 Genetic diversity among the genotypes

The objective of the experiment was to estimate the level of genetic diversity among the TGMS lines of rice using 16 SSRs markers. The UPGMA dendrogram was constructed using Jaccard's similarity coefficients based on SSRs markers data generated on 18 TGMS lines (Fig.4.4.2.7). A total of 47 alleles were amplified using 16 SSR primer pairs in the present analysis. All the lines except for marker RM499 were found to be polymorphic. The range of alleles was 2 - 5, while the average number of alleles per primer was 2.93. **Shah et al. (2012)** observed 5 to 17 alleles per locus with a mean of 9.4 alleles per locus.

Thirteen rare alleles, one of 180bp with primer RM154 in TGMS-1, 120bp with primer RM190 in TGMS-3 and TGMS-4, 150bp with primer RM276 in TGMS-10 and TGMS-17, 210bp and 310bp with primer RM287 in TGMS-6, 180bp and 200bp with primer RM279 in TGMS-1 and TGMS-18, 205bp with Rm327 in TGMS-16 and TGMS-18, 200bp with RM324 in TGMS-6 and TGMS-9, 300bp with RM418 in TGMS-5 and TGMS-7, 180bp with RM335 in TGMS-15, 300bp with RM424 in TGMS-5 and TGMS-7 and in the last 180bp with RM204 in TGMS-3 were identified, which can be used for identification and characterization of the genotypes. The polymorphic information content (PIC) for these 16 SSR primer pairs ranged from 0.1780 (RM327, RM418 and RM424) to 0.3750 (RM499) with mean value 0.2543 (RM204).

Table 4.20: Similarity coefficient between genotypes using sixteen SSR primers

Sl.No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1	1.00																	
2	0.74	1.00																
3	0.63	0.76	1.00															
4	0.68	0.68	0.69	1.00														
5	0.55	0.63	0.61	0.70	1.00													
6	0.59	0.55	0.48	0.57	0.53	1.00												
7	0.59	0.59	0.53	0.65	0.70	0.74	1.00											
8	0.53	0.61	0.68	0.68	0.72	0.72	0.72	1.00										
9	0.51	0.59	0.70	0.53	0.65	0.61	0.53	0.80	1.00									
10	0.57	0.61	0.59	0.68	0.68	0.55	0.63	0.74	0.76	1.00								
11	0.59	0.63	0.65	0.65	0.65	0.57	0.65	0.80	0.82	0.93	1.00							
12	0.57	0.65	0.68	0.63	0.68	0.55	0.63	0.82	0.85	0.91	0.97	1.00						
13	0.59	0.59	0.61	0.70	0.61	0.57	0.65	0.76	0.78	0.89	0.95	0.93	1.00					
14	0.63	0.59	0.57	0.61	0.53	0.61	0.57	0.68	0.74	0.80	0.87	0.85	0.91	1.00				
15	0.61	0.61	0.63	0.59	0.55	0.63	0.59	0.78	0.76	0.74	0.80	0.82	0.76	0.85	1.00			
16	0.65	0.61	0.55	0.63	0.63	0.51	0.46	0.61	0.68	0.74	0.72	0.74	0.68	0.76	0.78	1.00		
17	0.57	0.61	0.68	0.55	0.51	0.59	0.55	0.74	0.76	0.74	0.76	0.78	0.72	0.80	0.91	0.74	1.00	
18	0.59	0.55	0.53	0.57	0.48	0.57	0.53	0.63	0.65	0.72	0.78	0.76	0.82	0.91	0.76	0.76	0.72	1.00

UPRI-99-70-1 =1, UPRI99-71-1 =2, UPRI-99-71-2 =3, UPRI-99-73-1 =4, UPRI-99-73-2 =5, UPRI-99-73-3 =6, UPRI-99-73-4 =7, UPRI-99-74-3 =8, UPRI-99-79-1 =9, UPRI-97-60-1 =10, UPRI-99-72-1 =11, UPRI-99-72-3 =12, UPRI-99-72-4 =13, UPRI-99-74-1 =14, UPRI-99-74-3=15, UPRI-99-75-1=16, UPRI-99-78-1=17 and UPRI-97-60-8=18

The SSR diversity observed in the present study is comparable to those reported by **Nur Azreen *et al.* (2014)** total of 81 alleles with the 26 SSR markers, with an average of 3.12 alleles per locus and a PIC value varying from 0.028 to 0.450 in two thermosensitive genic male sterile (TGMS) lines. **Meti *et al.* (2013)** reported that a total of 28 alleles with 12 SSR primers in 48 aromatic rice varieties/landraces. The number of alleles per locus ranged from 1 to 5 with an average 2.08. Out of 28 bands, 25 bands were polymorphic and three were monomorphic bands. The results reveal that all the tested primers showed distinct polymorphism among the landraces/varieties indicating the robust nature of SSR markers. Most of the primers showed highest polymorphic information content (PIC). **Singh *et al.* (2011)** also reported 27 out of 30 markers to be polymorphic, amplifying a total of 83 alleles. Each SSR marker amplified 2–6 alleles with an average of 2.76 alleles per marker and a PIC value varying from 0.54 to 0.96. Morphological traits and SSR markers were used to determine the genetic relationship among 12 elite thermosensitive genic male sterile (TGMS) lines developed at three different research institutions of India. **Sajib *et al.* (2012)** observed that the number of alleles per locus ranged from 2 to 6, with an average of 3.33 alleles across 9 loci obtained in the study. The polymorphic information content values ranged from 0.14 to 0.71 in all 9 loci with an average of 0.48. A total of 24 SSR markers were used across 12 elite aromatic rice genotypes for their characterization and discrimination. **Matin *et al.* (2012)** observed total of 79 alleles with an average of 4.38 alleles per locus. The polymorphism information content (PIC) value ranged from 0.477 to 0.782, with an average of 0.634. The study was taken to assess the genetic diversity among deep water rice genotypes using Simple Sequence Repeat (SSR) markers through marker aided selection (MAS). **Ramadan *et al.* (2015)** reported that 2 to 6 alleles per locus with an average of 2.76 alleles. The overall size of amplified fragments ranged from 93 to 487 bp. The polymorphic information content (PIC) values ranged from 0.21 to 0.79 with an average of 0.46. Seven rice genotypes differing in drought tolerance were evaluated for genetic diversity by using 46 SSR markers related to drought.

4.4.5 Genotype clustering based on molecular data

The UPGMA (un-weighted pair group method with arithmetic mean) dendrogram was constructed using Jaccard's similarity coefficient of SSR marker data

of sixteen polymorphic primers generated on eighteen genotypes employing the program NTSYS 2.11.

The dendrogram constructed from SSR data divided eighteen TGMS lines in to three groups, I, II and III (Fig. 4.18).

The cluster I was formed at 0.59 Jaccard's coefficient of similarity. It consisted of two genotypes [UPRI-99-73-3 (6) and UPRI-99-73-4 (7)] clustered together at similarity coefficient 0.74. Group I related to Group II at similarity coefficient of 0.61.

Cluster II consisted of two sub-clusters; sub-cluster IIa and sub-cluster IIb. Sub-cluster IIa contained ten genotypes. Of these, UPRI-99-72-1 (11) and UPRI-99-72-3 (12) were not further separated indicating the high level of genetic similarity (0.97) between the two, i.e., some ancestral relationship seems to be there between UPRI-99-72-1 (11) and UPRI-99-72-3 (12).

Sub-cluster IIb consisted of only one genotypes UPRI-99-75-1 (16) which was related with sub-cluster IIa by similarity coefficient of 0.77. UPRI-99-75-1 (16) line was diverse from the other lines.

Cluster III consisted of five genotypes among these two lines UPRI-99-73-1 (4) and UPRI-99-73-2 (5) which were related to each other by similarity coefficient of 0.57. The maximum similarity coefficient observed between UPRI99-71-1 (2) and UPRI-99-71-2 (3) was 0.76. Cluster III related to cluster II at 0.65 similarity coefficient.

Based on the SSR marker clustering pattern no any correlation found with morphological characters.

Genetic diversity within the genus gives us an important source of variation that to be used to modify rice crop species by various methods. Estimates of genetic relationships are very important in designing crop improvement programmes, management of germplasm and evolution of conservation strategies. With the advent of recent methods in molecular biology, different molecular markers have been applied to the study of phylogenetic relationships and identification among and within the species.

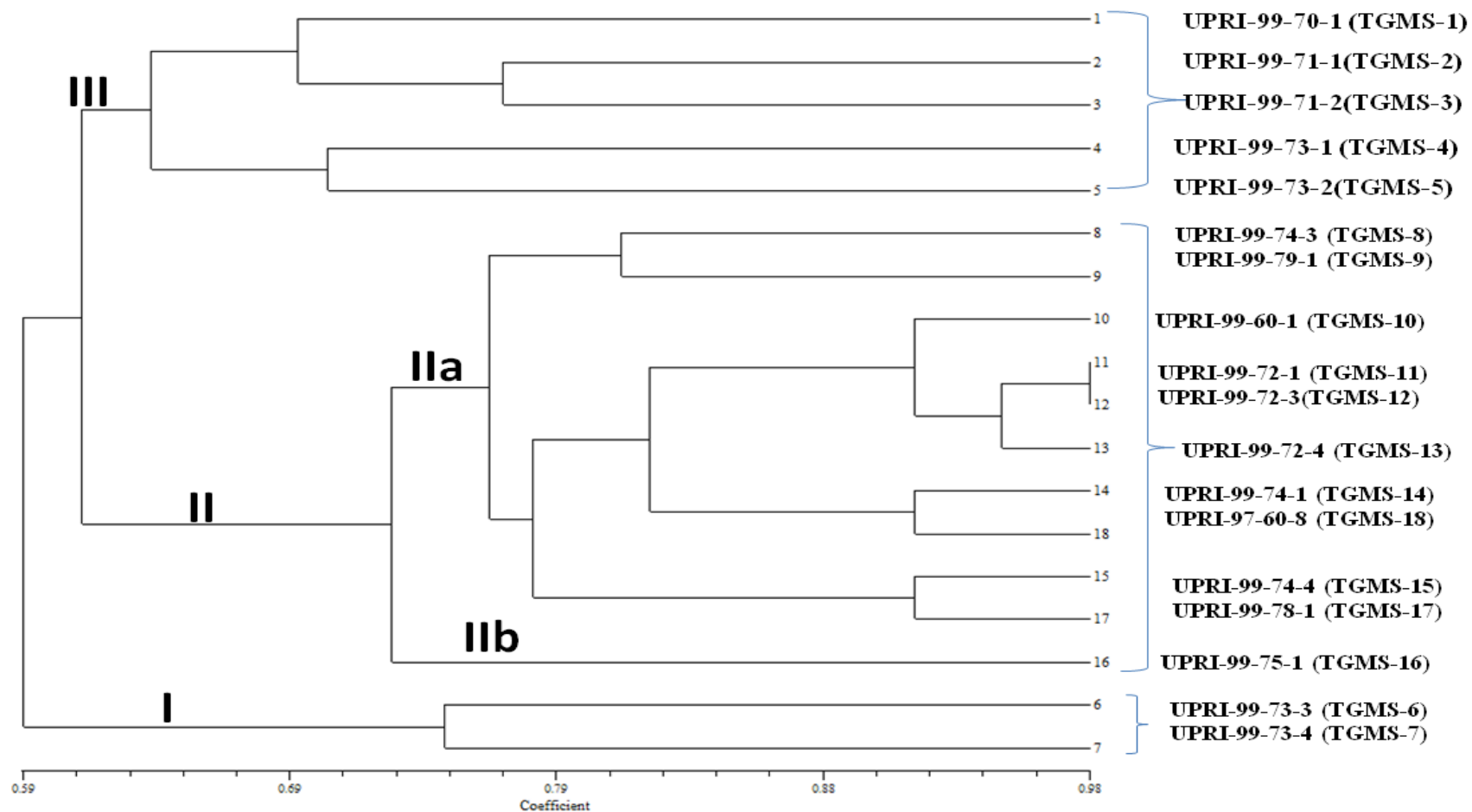
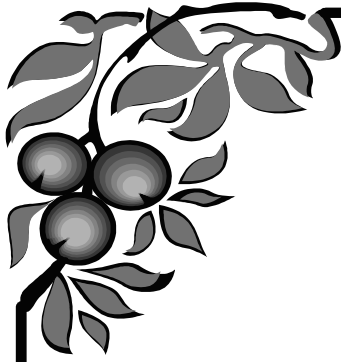


Figure 4.18: Dendrogram constructed of 18 TGMS lines

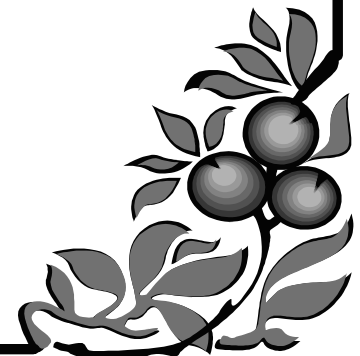
Table: 4.21 Association of TGMS lines fertility/sterility with marker data

Clusters	Sub-clusters	Lines	Spikeletes fertility	Markers	Specific bands
Cluster I		TGMS-6	Fertile	RM 324	200bp
		TGMS-7	Sterile		
Cluster II	Ila	TGMS-16	Sterile		
	Iib	TGMS-8	Sterile		
		TGMS-9	Fertile	RM 324	200bp
		TGMS-15	Sterile		
		TGMS-17	Sterile		
		TGMS-14	Sterile		
		TGMS-18	Fertile	RM 279	180bp and 200bp
		TGMS-10	Sterile		
		TGMS-13	Sterile		
		TGMS-11	Sterile		
		TGMS-12	Sterile		
Cluster III		TGMS-4	Sterile		
		TGMS-5	Sterile		
		TGMS-1	Fertile	RM 279	180bp and 200bp
				RM 154	180bp
		TGMS-2	Sterile		
		TGMS-3	Sterile		

Association study between the banding pattern of different markers and spikeletes fertility/sterility of TGMS lines (Table- 4.21) showed that four lines among the eighteen were fertile. All the three clusters contained one or two fertile line in each namely, cluster I (TGMS-6), cluster II (TGMS-9) and (TGMS-18) and cluster III (TGMS-1). These fertile lines separated to other sterile lines by three markers with unique bands. TGMS-6 and TGMS-9 showed 200bp specific band by RM 324 marker, TGMS-1 and TGMS-18 showed 180bp and 200bp specific bands and in TGMS-1 also showed 180bp specific band with RM 254 marker, it means that the specific bands 180bp and 200bp generated by different markers in different lines responsible for fertility. This molecular diversity analysis may be useful for identification of TGMS lines. Another association between marker and TGMS lines, study for gca of TGMS lines for number of traits, there were four TGMS lines good for different traits viz, TGMS-14, TGMS-17, TGMS-10 and TGMS-18. Among these lines only two lines showed specific band only by one marker that were TGMS-10 and TGMS-17 with 150bp specific band by RM 276 marker, this may be helpful for identification of good TGMS lines for number of traits and used in hybrid development for getting higher yield.



Summary and Conclusion



Chapter-5 SUMMARY AND CONCLUSION

The present investigation entitled “**Morphological and molecular evaluation of thermosensitive genetic male sterile (TGMS) lines and their heterotic combinations in rice (*Oryza sativa* L.)**” was undertaken with the following objectives:

- Determination of the critical sterility and critical fertility temperature for the different TGMS lines
- Evaluation and characterization of TGMS lines based on morphological traits and molecular markers
- To estimate the nature and magnitude of heterosis among of the crosses
- To estimate the nature of gene action and combining ability of TGMS lines and crosses for yield and other yield contributing traits.

The present investigation was carried out at the Borlaug Crop Research Center of G. B. Pant University of Agriculture and Technology, Pantnagar, Uttarakhand (India) during 2013 and 2014. The eighteen TGMS lines were evaluated for sterility and fertility in three seasons and two main seasons during 2013 and 2014 and off-season during 2014. The eighteen lines were crossed with four testers during *kharif* season in 2013 using Line X Tester mating design. The resulting seventy two F_1 's with twenty two parents (lines and testers) grown in randomized complete block design in three replications along with four checks.

The eighteen TGMS lines were characterized based on sixteen quantitative characters and molecular genetic diversity among these TGMS lines were also assessed under present study. The data were recorded for these TGMS lines viz, days to 50% flowering, panicle type, apiculous pigmentation, stigma color, awning, anthesis time, anthesis time duration (min.), plant height, glume angle, panicle exertion percent, stigma exertion percent, stem color, panicle lengths, pollen sterility, number of tillers per plant and plant height.

The data recorded on F_1 's were observed on randomly selected five plants from each plot of replication for Days to 50% flowering, Plant height, Panicle length, Panicle number per plant, Spikelet number per panicle, Grain number per panicle, Per cent spikelet fertility, 1000 grain weight, Harvest index and Grain yield per plant. Line X Tester analysis done without

parents and combining ability as suggested by Kempthorne (1957) through computer generated ENDOSTAT 3.0. The investigation also included the molecular diversity analysis of eighteen TGMS lines by sixteen SSR markers. The molecular work has been done at Pantnagar Centre for Plant Genetic Resources (PCPGR) lab and UG Plant Biotechnology Lab of the department of Genetics and Plant Breeding, Pantnagar.

The results of the present study have been concluded as under:

1. Combining ability and standard heterosis

- ❖ The lines that were best general combiner for different traits viz; TGMS-14 was best general combiner for days to 50% flowering, plant height, panicle length and grain yield per plant. TGMS-17 was good combiner for panicle length, spikelet number per panicle, grain number per panicle and harvest index. TGMS-10 was good combiner for plant height, percent spikelet fertility, 1000 grain weight and grain yield per plant and another promising line and TGMS-18 was good combiner for spikelet number per panicle, grain number per panicle, 1000 grain weight and grain yield per plant.
- ❖ The testers Pant Dhan-12 was the good combiner for days to 50% flowering, plant height, panicle number per plant, spikelet number per panicle, grain number per panicle and 1000 grain weight. Pant Dhan-4 for plant height, panicle number per plant, panicle length, harvest index and grain yield per plant UPRI-93-287R was good combiner for days to 50% flowering, plant height, panicle length and grain yield per plant.
- ❖ Association study between marker and GCA effects of TGMS lines, based on GCA there were four TGMS lines good for different traits viz, TGMS-14, TGMS-17, TGMS-10 and TGMS-18. Among these lines only two lines showed unique band only by one marker that were TGMS-10 and TGMS-17 at 150bp by RM 276 marker, this may be helpful for identification of good TGMS lines for number of traits and used in hybrid development for getting higher yield.
- ❖ Based on the SCA effect and *per se* performance the common crosses were selected for different traits. For days to 50% flowering viz. TGMS-16 × Pant Basmati-1, TGMS-14 × Pant Dhan-12 and TGMS-12 × UPRI-93-287R were the best hybrids For early maturity, TGMS-4 × Pant Dhan-4 and TGMS-11 × Pant Dhan-12 For dwarf plant type, For panicle number per plant viz. TGMS-4 × Pant Dhan-4 and TGMS-7 × UPRI-93-287R, For panicle length TGMS-12 × Pant Basmati-1, good hybrids For spikeletes number per panicle and grain number per panicle were TGMS-17 × Pant

Basmati-1 and TGMS-10 $\times$ Pant Dhan-4, For percent spikeletes fertility was TGMS-1 $\times$ UPRI-93-287R, For 1000 grain weight were TGMS-4 $\times$ Pant Dhan-4 and TGMS-15 $\times$ Pant Dhan-4, For harvest index TGMS-16 $\times$ Pant Basmati-1 and TGMS-18 $\times$ Pant Dhan-4 and For grain yield per plant good hybrids were TGMS-14 $\times$ Pant Dhan-4, TGMS-9 $\times$ Pant Basmati-1 and TGMS-16 $\times$ UPRI-93-287R.

- ❖ The study of crosses in relation to GCA effects indicated high $\times$ high, high $\times$ low, high $\times$ average, low $\times$ average, low $\times$ high, low $\times$ low, average $\times$ high, average $\times$ low and average $\times$ average GCA combinations of the parents. It could, therefore, be concluded that allelic and non-allelic interactions were involved in the inheritance of these traits.
2. Based on the significant and high estimates of standard heterosis five crosses for seed yield were identified which also showed high SCA effect and *per se* performance. TGMS-14 $\times$ Pant Dhan-4, TGMS-16 $\times$ UPRI-93-287R, TGMS-17 $\times$ UPRI-93-287R, TGMS-9 $\times$ Pant Basmati-1 and TGMS-14 $\times$ UPRI-93-287R. All the five crosses emerged from the High $\times$ High GCA effect of parents except one cross which was showed High $\times$ Low GCA effect.

3. Component of genetic variance and gene action

- ❖ Estimates of variance of general combining ability were lower than those of variance of specific combining ability for all the traits. Showing preponderance of non-additive gene action except plant height, panicle length and spikelet number per panicle which showing additive gene action.
- ❖ High estimates of broad sense heritability coupled with high genetic advance for spikelet number per panicle. For yield high heritability coupled with moderate genetic advance indicated the scope of getting better recombinants.
- ❖ The contribution of lines to the total variance was greater than testers and line $\times$ tester interaction for three characters, viz. days to flowering, spikelet number per panicle and grain yield per plant indicating predominant maternal influence for these traits. Contribution of testers was more than lines and line $\times$ tester interactions for plant height and panicle length. Line $\times$ tester interactions contributed more than lines and testers for Panicle Number per Plant, Grain Number per Panicle, Percent Spikelet Fertility, 1000 Grain Weight and harvest index.

4. Evaluation of TGMS lines

- ❖ There were different lines found suitable for different traits. The lines which showed high panicle exertion, high stigma exertion, more anthesis duration and high pollen sterility could be useful in two line hybrid development.
- ❖ The lines found suitable for high panicle exertion TGMS-3, TGMS-4, TGMS-5, TGMS-8 and TGMS-18, for high stigma exertion were TGMS-11 and TGMS-17, the line with high anthesis duration were TGMS-8, TGMS-3 and TGMS-11 and the lines with high pollen sterility were TGMS-4, TGMS-5, TGMS-9 and TGMS-12. These lines can be used in two line hybrid development.
- ❖ Out of eighteen lines, ten TGMS lines viz, TGMS-2, TGMS-3, TGMS-4, TGMS-5, TGMS-7, TGMS-8, TGMS-10, TGMS-11, TGMS-14 and TGMS-15 appeared complete sterile at average temperature 28.53 to 28.92°C. These can be used to develop the two line hybrid in rice.
- ❖ Out of eighteen lines, four lines TGMS-1, TGMS-6, TGMS-9 and TGMS-18 showed fertile spikelets in both main *kharif* season during 2013 and 2014. But showed complete sterility in 2014 offseason. Its meaning that lines required high temperature compare to main season because in offseason the average temperatures were 31.74 to 31.94°C. So these lines required >29°C critical temperature for complete sterility. Another four lines viz. TGMS-12, TGMS-13, TGMS-16 and TGMS-17 found that seed setting in *kharif*, 2013 and complete sterility in *kharif* and offseason 2014. It is meaning that the critical sterility temperature for these lines slightly higher 29.39-29.53°C than the 2013 *kharif* season.
- ❖ Therefore in Pantnagar situation for getting complete sterility these lines sowing will be done in last week of May and transplanting will be done after 25 to 30 days seedlings and then we can go for two line hybrid programme.

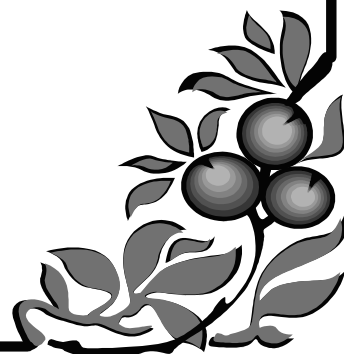
5. Molecular genetic diversity

- ❖ A total of 47 alleles were amplified using 16 SSR primer pairs in the present analysis of eighteen rice TGMS lines all of which were found to be polymorphic except for one primer pair. The range of alleles was 2 - 5 while the average number of alleles per primer was 2.93.

- ❖ Thirteen rare alleles found, one of 180bp with primer RM154 in TGMS-1, 120bp with primer RM190 in TGMS-3 and TGMS-4, 150bp with primer RM276 in TGMS-10 and TGMS-17, 210bp and 310bp with primer RM287 in TGMS-6, 180bp and 200bp with primer RM279 in TGMS-1 and TGMS-18, 205bp with Rm327 in TGMS-16 and TGMS-18, 200bp with RM324 in TGMS-6 and TGMS-9, 300bp with RM418 in TGMS-5 and TGMS-7, 180bp with RM335 in TGMS-15, 300bp with RM424 in TGMS-5 and TGMS-7 and in the last 180bp with RM204 in TGMS-3 were identified, which can be used for identification and characterization of the genotypes.
- ❖ The polymorphic information content (PIC) for these sixteen SSR primer pairs ranged from 0.1780 (RM327, RM418 and RM424) to 0.3750 (RM499) with mean value 0.2543 (RM204). This indicated that SSR markers could be efficiently applied to detect polymorphism even with relatively low number of alleles.
- ❖ The range of Jaccard's similarity between genotype pair was found to vary from maximum between UPRI-99-72-1 (11) and UPRI-99-72-3 (12) to a minimum between UPRI-99-73-4 (7) and UPRI-99-75-1 (16).
- ❖ Association among the genotypes revealed as expressed by Unweighted Pair Group Method with Arithmetic Mean (UPGMA) cluster analysis. The dendrogram constructed from SSR marker data divided eighteen rice TGMS lines into three clusters: Cluster I, II and III. Cluster I contained two line, Cluster II eleven lines and Cluster III contained five lines.
- ❖ Association study between the banding pattern of different markers and spikeletes fertility/sterility of TGMS lines showed that four lines out of eighteen were fertile. All the three clusters contained one or two fertile line, cluster I (TGMS-6), cluster II (TGMS-9) and (TGMS-18) and cluster III (TGMS-1). These fertile lines separated to other sterile lines by three markers with unique bands. TGMS-6 and TGMS-9 showed 200bp specific band by RM 324 marker, TGMS-1 and TGMS-18 showed 180bp and 200bp unique bands and in TGMS-1 also showed 180bp unique band with RM 254 marker, it means that the unique bands 180bp and 200bp generated by different markers in different lines responsible for fertility. This molecular diversity analysis may be useful for identification of TGMS lines or may be used for marker assisted selection (MAS) for two line hybrid development.



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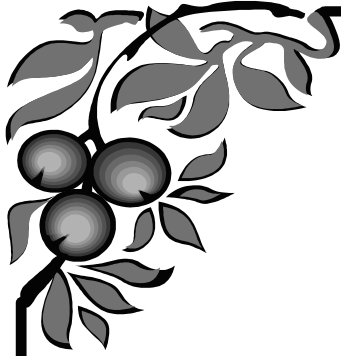
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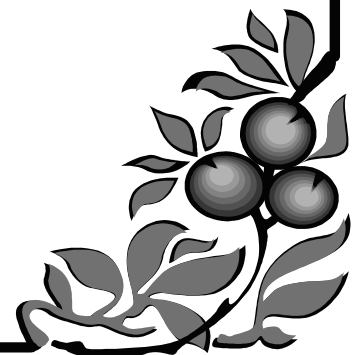
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Appendices



APPENDICES

Appendix-I

Table-1: Mean table for different traits in F₁

S.No.	Character	Days to 50% Flowering	Plant Height	Panicle Number Per Plant	Panicle Length	Spikelets Number Per Panicle	Grain Number Per Panicle	Percent Spikeletes Fertility	1000 Grain Weight	harvest Index	Grain Yield Per Plant
1.	TGMS-1*Pant Basmati-1	102.33	112.40	12.20	26.64	164.40	130.60	79.44	22.60	42.85	33.60
2.	TGMS-1*Pant Dhan-4	106.33	111.58	15.20	27.36	262.40	186.00	70.88	26.22	37.39	36.80
3.	TGMS-1*Pant Dhan-12	99.67	111.52	11.20	26.12	212.60	185.60	87.30	25.82	55.21	36.00
4.	TGMS-1*UPRI-93-287-R	114.00	112.48	11.20	28.20	197.00	192.40	97.66	26.36	48.18	37.20
5.	TGMS-2* Pant Basmati-1	101.33	113.42	10.00	29.00	194.80	174.60	89.63	21.64	49.76	41.60
6.	TGMS-2*Pant Dhan-4	96.33	111.10	8.60	28.80	237.00	198.40	83.71	25.72	51.46	35.20
7.	TGMS-2*Pant Dhan-12	91.67	110.02	10.00	27.22	169.80	146.40	86.21	25.28	50.82	36.80
8.	TGMS-2*UPRI-93-287-R	99.33	108.12	9.80	26.76	184.40	176.20	95.55	26.12	55.21	36.00
9.	TGMS-3* Pant Basmati-1	101.33	113.36	9.20	30.60	224.80	187.00	83.18	25.78	53.29	38.80
10.	TGMS-3*Pant Dhan-4	94.00	106.98	12.00	28.02	229.40	211.40	92.15	27.72	59.02	41.20
11.	TGMS-3*Pant Dhan-12	98.67	111.04	13.80	26.08	211.40	184.00	87.03	22.74	56.35	40.80
12.	TGMS-3*UPRI-93-287-R	103.00	105.18	9.60	25.56	172.40	158.40	91.87	24.00	49.68	31.20
13.	TGMS-4* Pant Basmati-1	117.33	114.96	13.60	29.54	192.60	174.40	90.55	21.32	53.88	45.80
14.	TGMS-4*Pant Dhan-4	120.00	100.42	21.40	28.02	203.40	182.80	89.87	30.58	43.13	44.00
15.	TGMS-4*Pant Dhan-12	103.67	107.34	17.20	29.30	176.00	159.00	90.34	27.70	46.26	39.60
16.	TGMS-4*UPRI-93-287-R	100.67	111.04	12.60	26.90	184.20	145.60	79.04	23.04	43.55	50.00
17.	TGMS-5* Pant Basmati-1	105.33	115.64	12.00	30.04	180.60	170.00	94.13	23.92	50.88	40.20
18.	TGMS-5*Pant Dhan-4	112.00	111.98	16.00	28.62	202.00	173.40	85.84	26.74	47.61	52.00
19.	TGMS-5*Pant Dhan-12	103.00	112.66	14.00	28.72	161.80	130.40	80.59	27.68	51.42	36.00
20.	TGMS-5*UPRI-93-287-R	105.67	112.16	12.60	28.24	168.60	153.20	90.86	24.12	50.00	48.80

21.	TGMS-6* Pant Basmati-1	101.33	114.12	9.80	30.32	179.40	162.00	90.30	23.78	48.38	45.00
22.	TGMS-6*Pant Dhan-4	103.33	108.18	10.00	28.58	232.00	204.80	88.27	25.40	59.21	60.40
23.	TGMS-6*Pant Dhan-12	99.00	107.34	14.20	26.20	157.00	139.20	88.66	26.28	53.64	50.00
24.	TGMS-6*UPRI-93-287-R	110.00	108.82	11.60	26.50	234.80	204.60	87.13	23.00	44.19	39.60
25.	TGMS-7* Pant Basmati-1	108.67	116.14	10.80	30.92	196.00	179.00	91.32	20.58	51.45	49.60
26.	TGMS-7*Pant Dhan-4	98.33	103.42	10.40	28.84	217.40	197.60	90.89	25.80	58.79	54.80
27.	TGMS-7*Pant Dhan-12	102.67	104.64	11.40	26.98	169.40	153.80	90.79	22.94	43.47	40.00
28.	TGMS-7*UPRI-93-287-R	100.00	107.04	17.40	26.90	164.20	137.60	83.80	23.84	50.34	57.60
29.	TGMS-8* Pant Basmati-1	98.00	118.20	12.00	29.12	168.20	156.00	92.74	24.00	55.39	45.20
30.	TGMS-8*Pant Dhan-4	98.33	108.20	14.80	26.44	208.00	168.40	80.96	24.30	46.40	46.40
31.	TGMS-8*Pant Dhan-12	108.33	107.88	10.60	26.50	159.20	150.20	94.34	26.00	53.99	46.00
32.	TGMS-8*UPRI-93-287-R	92.67	114.24	13.60	25.48	166.40	158.40	95.19	25.06	48.86	43.20
33.	TGMS-9* Pant Basmati-1	102.00	122.36	12.00	30.94	230.60	210.80	91.41	24.64	55.07	71.60
34.	TGMS-9*Pant Dhan-4	98.00	104.20	11.20	26.56	257.60	218.80	84.93	26.72	45.26	43.00
35.	TGMS-9*Pant Dhan-12	96.33	112.72	11.00	28.58	195.00	171.40	87.89	24.36	50.12	39.60
36.	TGMS-9*UPRI-93-287-R	90.00	106.58	9.80	27.20	181.60	166.60	91.74	24.98	48.54	46.80
37.	TGMS-10* Pant Basmati-1	100.67	114.64	10.60	30.04	184.00	172.80	93.91	22.14	59.11	53.20
38.	TGMS-10*Pant Dhan-4	99.67	107.24	11.80	29.88	270.00	243.20	90.07	25.16	63.22	56.40
39.	TGMS-10*Pant Dhan-12	98.67	103.30	13.20	27.56	151.00	137.20	90.86	24.64	61.22	48.00
40.	TGMS-10*UPRI-93-287-R	96.00	103.54	11.80	27.38	171.00	165.20	96.60	25.62	53.08	51.60
41.	TGMS-11* Pant Basmati-1	106.67	130.24	10.80	31.44	209.00	179.60	85.93	22.26	51.66	49.60
42.	TGMS-11*Pant Dhan-4	115.00	102.96	10.80	28.30	259.60	219.20	84.43	26.88	47.22	46.00
43.	TGMS-11*Pant Dhan-12	99.00	101.34	11.80	28.88	191.00	159.40	83.45	22.38	40.91	41.00
44.	TGMS-11*UPRI-93-287-R	100.33	113.82	14.00	29.12	176.40	162.80	92.29	24.04	54.60	61.60
45.	TGMS-12* Pant Basmati-1	102.00	122.18	11.00	32.40	201.40	162.40	80.63	23.14	49.41	50.40
46.	TGMS-12*Pant Dhan-4	102.00	102.24	12.00	28.34	255.60	208.80	81.69	27.20	50.39	51.20
47.	TGMS-12*Pant Dhan-12	99.33	102.30	11.60	28.06	195.40	167.80	85.87	23.56	44.44	44.00
48.	TGMS-12*UPRI-93-287-R	89.67	111.06	12.00	28.98	190.60	164.00	86.04	26.12	51.11	55.20
49.	TGMS-13* Pant Basmati-1	90.33	115.62	11.00	29.76	188.00	148.00	78.72	21.90	64.07	51.00
50.	TGMS-13*Pant Dhan-4	92.00	109.08	9.20	28.82	256.60	218.40	85.11	23.84	60.00	45.60
51.	TGMS-13*Pant Dhan-12	94.00	104.36	12.80	26.88	173.60	158.60	91.35	24.86	57.66	57.20
52.	TGMS-13*UPRI-93-287-R	93.33	110.70	12.20	28.16	201.20	172.40	85.68	24.52	53.70	52.20

53.	TGMS-14* Pant Basmati-1	104.67	119.16	9.60	31.76	168.60	148.20	87.90	25.46	50.00	42.80
54.	TGMS-14*Pant Dhan-4	100.00	106.10	15.40	29.50	208.80	195.40	93.58	26.04	59.88	82.40
55.	TGMS-14*Pant Dhan-12	86.67	100.16	11.00	29.22	156.80	129.20	82.39	25.88	53.42	46.80
56.	TGMS-14*UPRI-93-287-R	96.67	103.48	13.00	29.86	146.00	135.20	92.60	27.46	54.78	66.40
57.	TGMS-15* Pant Basmati-1	108.00	132.04	14.20	31.14	243.20	193.60	79.60	26.26	32.20	38.00
58.	TGMS-15*Pant Dhan-4	103.67	116.52	11.40	31.02	215.40	180.00	83.56	29.34	54.25	53.60
59.	TGMS-15*Pant Dhan-12	103.67	109.98	12.20	29.30	202.20	168.80	83.48	21.24	51.47	42.00
60.	TGMS-15*UPRI-93-287-R	98.00	112.14	11.40	27.04	238.60	201.60	84.49	23.52	45.36	45.00
61.	TGMS-16* Pant Basmati-1	81.67	108.84	12.60	28.42	157.00	133.80	85.22	23.80	68.08	64.00
62.	TGMS-16*Pant Dhan-4	88.67	116.02	13.80	27.70	158.00	139.40	88.22	26.24	54.41	46.80
63.	TGMS-16*Pant Dhan-12	93.67	110.28	13.00	28.98	192.80	164.20	85.16	24.56	49.79	49.60
64.	TGMS-16*UPRI-93-287-R	99.67	114.34	13.60	27.34	190.00	181.40	95.47	26.40	54.04	74.80
65.	TGMS-17* Pant Basmati-1	102.33	121.46	9.60	31.18	290.00	235.40	81.17	24.80	54.22	61.60
66.	TGMS-17*Pant Dhan-4	101.00	108.68	8.60	29.70	284.40	212.20	74.61	26.86	54.98	55.20
67.	TGMS-17*Pant Dhan-12	88.67	109.04	12.60	29.26	214.80	187.00	87.05	24.25	57.61	56.00
68.	TGMS-17*UPRI-93-287-R	97.00	112.90	14.00	28.48	208.40	179.40	86.06	22.78	54.70	74.40
69.	TGMS-18* Pant Basmati-1	91.67	118.22	13.40	29.76	211.60	182.20	86.10	25.86	50.00	50.40
70.	TGMS-18*Pant Dhan-4	103.00	114.60	10.00	29.48	275.20	203.80	74.05	29.94	62.39	58.40
71.	TGMS-18*Pant Dhan-12	99.67	108.84	10.80	26.50	225.40	178.40	79.14	24.02	52.07	65.20
72.	TGMS-18*UPRI-93-287-R	99.33	109.04	10.20	27.36	204.80	190.00	92.77	25.22	51.19	60.00
	Mean	100.12	110.89	12.08	28.51	201.57	174.69	87.10	24.93	51.88	49.00
	C.V.	2.02	2.61	2.50	2.56	2.64	2.69	2.55	2.66	2.69	2.79
	F ratio	35.56	13.58	159.20	14.27	125.82	91.48	18.80	26.92	58.08	175.06
	F Prob.	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	S.E.	1.17	1.67	0.17	0.42	3.07	2.71	1.28	0.38	0.81	0.79
	C.D. 5%	3.26	4.68	0.49	1.18	8.59	7.58	3.58	1.07	2.26	2.20
	C.D. 1%	4.30	6.18	0.64	1.55	11.35	10.02	4.73	1.41	2.98	2.91
	Range Lowest	81.67	100.16	8.60	25.48	146.00	129.20	70.88	20.58	32.20	31.20
	Range Highest	120.00	132.04	21.40	32.40	290.00	243.20	97.66	30.58	68.08	82.40

Appendix-II (a)

Table -2: Mean weekly temperature (⁰C), 2013

Month	Week Number	Period & Date	Temperature (⁰ C)		
			Maximum	Minimum	Mean
April, 2013	1	1-7	33.10	14.95	24.02
	2	8-14	36.84	17.70	27.32
	3	15-21	33.31	20.42	26.87
	4	22-28	34.05	19.25	26.65
	5	29-5	38.65	18.80	28.72
May, 2013	6	6-12	39.62	20.05	29.84
	7	13-19	37.50	22.55	29.95
	8	20-26	38.77	28.87	33.90
	9	27-2	38.54	26.01	32.27
June, 2013	10	3-9	34.78	27.02	30.90
	11	10-16	33.78	25.75	29.77
	12	17-23	33.10	25.35	29.23
	13	24-30	30.98	25.12	28.02
July, 2013	14	1-7	32.87	25.50	29.18
	15	8-14	32.27	26.00	29.13
	16	15-21	31.05	25.74	28.40
	17	22-28	32.20	25.98	29.09
	18	29-4	32.64	26.14	29.39
August, 2013	19	5-11	32.55	25.24	28.87
	20	12-18	31.57	24.74	28.15
	21	19-25	33.44	25.87	29.65
	22	26-2	32.40	25.25	28.82
September, 2013	23	3-9	32.08	23.35	27.69
	24	10-16	33.52	23.88	28.70
	25	17-23	32.87	24.28	27.82
	26	24-30	31.11	25.38	28.25
October, 2013	27	1-7	30.50	23.17	26.83
	28	8-14	31.14	22.00	26.57
	29	15-21	30.32	19.28	24.91
	30	22-28	29.91	16.25	23.46

Appendix-II (b)

Table -3: Mean weekly temperature (⁰C), 2014

Month	Week Number	Period & Date	Temperature (⁰ C)		
			Maximum	Minimum	Mean
April, 2014	1	1-7	31.82	14.75	23.32
	2	8-14	33.42	15.14	24.28
	3	15-21	32.35	15.81	24.08
	4	22-28	36.84	17.20	26.95
	5	29-5	37.91	19.75	28.83
May, 2014	6	6-12	36.54	21.12	28.83
	7	13-19	36.14	20.35	28.25
	8	20-26	39.00	22.28	30.64
	9	27-2	37.22	25.42	31.32
June, 2014	10	3-9	40.52	26.21	33.37
	11	10-16	39.04	25.65	32.34
	12	17-23	34.82	26.24	30.55
	13	24-30	37.41	26.72	32.07
July, 2014	14	1-7	32.72	25.17	28.97
	15	8-14	33.10	26.72	29.91
	16	15-21	30.10	25.51	27.80
	17	22-28	33.24	26.40	29.82
	18	29-4	33.71	25.85	29.78
August, 2014	19	5-11	32.81	26.25	29.52
	20	12-18	32.22	25.50	28.86
	21	19-25	34.05	26.02	30.04
	22	26-2	34.15	25.71	29.93
September, 2014	23	3-9	33.04	25.24	29.14
	24	10-16	32.25	23.75	28.00
	25	17-23	33..10	23.41	28.25
	26	24-30	32.60	21.58	27.12
October, 2014	27	1-7	31.95	23.67	27.60
	28	8-14	32.90	18.11	25.50
	29	15-21	28.52	16.01	22.27
	30	22-28	29.72	16.77	23.27

Appendix-III (a)

Reagents for Genomic DNA isolation and PCR amplification

I. Reagents for Genomic DNA isolation

Requirements:

Tris base, EDTA- Na_2 , NaCl, Potassium Acetate, Glacial acetic acid, isopropanol, chloroform, isoamyl alcohol, and Absolute alcohol.

Preparation of solutions:

STOCKS SOLUTIONS:

1. 1M Tris-Cl buffer (pH 8.0), 100 ml

12.114 g Tris-base was dissolved in 80 ml ddw. The pH was adjusted to 8.0 by 6 N HCl. The volume was made upto 100 ml with ddw. Autoclaved and stored at 4°C.

2. 0.5 M EDTA (pH 8.0), 50 ml

9.3 g EDTA- Na_2 was dissolved in 30 ml of ddw (10N NaOH was added to make the pH 8.0). The final vol. was made upto to 50 ml, Autoclaved and stored at 4°C.

3. 10N NaOH, 50 ml

20 g of NaOH was dissolved in 30 ml of autoclaved ddw. The volume was made upto 50 ml. Stored in a plastic bottle at RT.

4. 5 M NaCl, 50 ml

14.6 g NaCl was dissolved in 30 ml of ddw. The final vol. was made upto 50 ml. Autoclaved and stored at RT.

WORKING SOLUTIONS

1. DNA extraction buffer 25 ml

2% (w/v) CTAB	:	0.5 g
100 mM Tris-Cl	:	2.5 ml (1M stock)
1.4 M NaCl	:	2.0475 g
20 mM EDTA	:	1 ml (0.5 M stock)
0.2 % beta mercaptoethanol	:	50 μl

The final vol. was made upto to 25 ml with ddw.

2. 5 M potassium Acetate (pH 5.4) 50 ml

24.8 g potassium acetate was dissolved in 30 ml of ddw. The pH was adjusted to 5.4 with glacial acetic acid. The final volume was made up to 50 ml with ddw. Autoclaved and stored at RT.

- | | | |
|---------------------------------------|---|---------------------|
| 3. Isopropanol | : | Kept at -20°C. |
| 4. Chloroform: isoamyl alcohol | : | 24:1, 25 ml |
| 5. 70% ethanol | : | 10 ml |
| 6. TE Buffer (pH 8.0) | | 25 ml |
| 10 mM Tris-Cl | : | 250 µl (1M stock) |
| 1mM EDTA | : | 50 µl (0.5 M stock) |

Volume was made up by adding ddw to 25 ml. Autoclaved and stored at RT.

II. PCR Ingredients

(i) Design and Synthesis of the primers

The most essential requirement of PCR is the availability of short oligonucleotides called primers having sequence complementary to either ends of the target DNA segment called template DNA to be synthesized. The primers used in the study are synthesized from Eurofins, Bangalore

(ii) Taq DNA polymerase

Taq DNA polymerase is a thermostable enzyme that replicates DNA at 72-74°C and remains functional even after incubation at 95°C. The enzyme has 5'-3' polymerase and 3'-5' exonuclease activity. The concentration of the enzyme was 3units per µl (3U/ µl).

(iii) dNTPs

The dNTPs used in this reaction were obtained from Genei Pvt. Ltd. Bangalore as 10mM each (dATP, dGTP, dCTP, dTTP).

(iv) Assay Buffer (10X)

Assay buffer (10X) contained 10 mM Tris-Cl (pH 9.0), 15 mM MgCl₂, 50 mM KCl and 0.01% gelatin.

III. Running buffer, Dye and Reagents

Running Buffer

0.5 X TBE buffer

Dye

98% formamide, 10mM EDTA, 0.023mg Bromophenol Blue, 0.023mg Xylene Cynol

Reagents used and preparation

1. Electrophoresis buffer (5X)	100ml
Tris base (0.045M)	54 g
Boric acid	27.5g
EDTA (0.001M)	10ml (1M stock)

Components were dissolved in 80ml of de-ionized water. pH was adjusted to 8.0 with 6N. Final volume was made up to 10 ml, autoclaved and stored at 4⁰C. 0.5X was the working solution of TBE buffer.

2. DNA loading dye (Double Dye)	10ml
Bromophenol Blue (0.25% w/v)	0.025g
Xylenecynol FF (0.25% w/v)	0.025g
Sucrose (40% w/v)	

The components were dissolved in 8.0ml of sterile de-ionized water. pH was adjusted to 8.0 and finally volume was made 10ml. Aliquots were made and stored at -20⁰C.

3. DNA staining solution and Ethidium Bromide (10000X)	
Ethidium Bromide	10ml
Sterile de-ionized water	1ml

Note:

- Working solution for staining gel was made by dissolving 60µl ethidium bromide Stock (10mg/ml) in 3000ml of de-ionized water.
- Stock was stored at 4⁰C
- Ethidium bromide being highly carcinogenic was handled wearing gloves.

Appendix-III (b)

Table-4: Scoring of molecular markers in TGMS lines based on the 0 and 1

Primers	size (bp)	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
RM-154																			
	210	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0
	200	0	1	1	0	1	0	0	1	1	1	1	1	0	0	1	1	1	0
	190	0	0	0	1	0	0	0	0	0	0	0	0	1	1	0	0	0	1
	180	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
RM-190	140	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1
	120	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	110	1	1	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0
RM-276	190	0	1	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
	150	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0
	110	1	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1
RM-336	190	0	1	1	1	1	0	1	1	1	1	1	1	1	0	0	0	0	0
	180	0	0	0	0	0	1	0	0	0	0	0	0	0	1	1	1	1	1
RM-287	310	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
	210	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
	125	0	0	0	0	1	1	0	1	1	0	0	0	0	0	0	0	0	0
	120	1	1	1	1	0	0	1	0	0	1	1	1	1	1	1	1	1	1
RM-279	200	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
	195	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1	0
	190	0	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0
	180	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
RM-327	210	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	0
	205	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1
RM-324	200	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0
	195	0	0	0	1	1	0	1	1	0	1	1	1	1	0	0	0	0	0
	190	1	1	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1
RM-341	180	0	0	0	0	1	0	0	0	1	1	1	1	1	1	0	1	0	1
	160	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	140	0	0	0	1	0	1	1	1	0	0	0	0	0	0	1	0	1	0
RM-166	305	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	300	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	290	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1
RM-418	300	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0
	295	1	1	1	1	0	1	0	1	1	1	1	1	1	1	1	1	1	1

RM-335	180	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
	160	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0
	140	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0
	110	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0
	100	0	1	0	0	0	0	0	0	0	1	1	1	1	1	0	0	0	1
RM-499	100	0	1	1	0	1	0	0	1	1	0	0	1	0	0	1	1	1	0
RM-424	300	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0
	295	1	1	1	1	0	1	0	1	1	1	1	1	1	1	1	1	1	1
RM-592	380	0	0	1	0	0	0	0	1	1	0	1	1	1	1	1	0	1	1
	320	0	0	0	1	1	0	0	0	0	1	0	0	0	0	0	1	0	0
	300	1	1	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0
RM-204	200	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	0	1	1
	180	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

VITA

Pardeep Kumar, the author of this manuscript was born on 20 August 1988, at district Saharanpur of Uttar Pradesh, India. He has passed his High School and Intermediate from G.K.I.C. Rampur Maniharan, Saharanpur. He completed B.Sc. (Ag.) from C.C.S. University, Meerut in 2009 and secured highest marks, got first rank and gold medal. He further completed M.Sc. (Ag.) in Genetics & Plant Breeding from C.S.A. University of Agriculture & Technology, Kanpur U.P. in 2012. He joined G. B. Pant University of Agriculture and Technology, Pantnagar for Ph.D. with major in Genetics and Plant Breeding and minor in Molecular Biology and Biotechnology in August 2012. After completing Master degree he qualified ICAR-ARS NET two times in 2012 and 2013.

Along with this he qualified ICAR-ARS in 2013 and joined on 1st January 2015.

Address for correspondence:

*Pardeep Kumar
S/o Shri Rampal Singh
Vill + Post Ghathera
Distt. Saharanpur, Pin- 247451(U.P.)
Mob.No.- 09756436828
E-mail: pardeepkumar656@gmail.com*

Name: Pardeep Kumar

Id.No. : 44160

Sem. & year of admission: Ist, 2012

Degree: Ph.D.

Major: Genetics and Plant Breeding

Department: Genetics and Plant Breeding

Minor: Molecular Biology and Biotechnology

Thesis title: Morphological and molecular evaluation of thermosensitive genetic male sterile (TGMS) lines and their heterotic combinations in rice (*Oryza sativa* L.)

Advisor: Dr. M. K. Nautiyal

ABSTRACT

The present investigation was taken up to evaluate the eighteen TGMS lines for different morphological traits and critical sterility/fertility temperature of TGMS lines, the molecular diversity analysis was based on the SSR primers of these lines. These eighteen TGMS lines were crossed with four testers and identified the best crosses for different yield and yield contributing traits. The field experiment conducted with eighteen TGMS lines, seventy two F₁'s and four checks in randomized complete block design with three replications at the Norman E. Borlaug Crop Research Centre of Govind Ballabh Pant University of Agriculture and Technology, Pantnagar during Kharif 2013 and Kharif 2014.

The Analysis of variance was found significant for all the characters in crosses and line x tester effect. For lines all the traits showed significant difference except plant height, percent spikelets fertility and 1000 grain weight and for tester the significant difference showed by the plant height, panicle length, spikelet number per panicle, grain number per panicle, percent spikelet fertility and 1000 grain weight. Based on the general combining ability among the lines the four lines TGMS-14, TGMS-17, TGMS-10 and TGMS-18 were best general combiner for number of characters and among the testers Pant Dhan-4, Pant Dhan-12 and UPRI-93-287R were good general combiner for different traits. Based on the marker association study, among these lines only two lines showed unique band by one marker that were TGMS-10 and TGMS-17 with 150bp unique band by RM 276 marker. This may be helpful for identification of good TGMS lines for number of traits and used in hybrid development for getting higher yield. Based on the sca effect and *per se* performance the common crosses were selected for different traits. The best three crosses for grain yield per plant viz. TGMS-14 × Pant Dhan-4, TGMS-9 × Pant DRR Basmati-1 and TGMS-16 × UPRI-93-287R these may be used for hybrid development. Based on the standard heterosis five economic crosses viz. TGMS-14 × Pant Dhan-4, TGMS-16 × UPRI-93-287R, TGMS-17 × UPRI-93-287R, TGMS-9 × Pant Basmati-1 and TGMS-14 × UPRI-93-287R for grain yield per plant was reported which showed high sca effect and *per se* performance. Estimates of variance of general combining ability were lower than those of variance of specific combining ability for all the traits showing preponderance of non-additive gene action except panicle length and spikelet number per panicle which showed additive gene action. High estimates of broad sense heritability coupled with high genetic advance for Spikelet number per panicle. For grain yield per plant high heritability coupled with moderate genetic advance indicated the scope of getting better recombinants.

Result on morphological and molecular characterization of TGMS lines showed that, not a single line was observed good for all the traits, different lines good for different traits. Among the eighteen TGMS lines ten lines viz. TGMS-2, TGMS-3, TGMS-4, TGMS-5, TGMS-7, TGMS-8, TGMS-10, TGMS-11, TGMS-14 and TGMS-15 showed complete sterility at average temperature 28.53 to 28.92°C. Therefore, in Pantnagar situation to get complete sterility these lines should be sown in last week of May. These can be used in hybrid development. Based on the molecular genetic diversity data indicated that total of 47 alleles were amplified using sixteen SSR markers in the present study of eighteen TGMS lines all were found polymorphic except for one marker. The range of alleles was 2 - 5, while the average number of alleles per primer was 2.93 and thirteen rare alleles found in different lines. The polymorphic information content (PIC) for these sixteen SSR markers ranged from 0.1780 to 0.3750 with mean value 0.2543. The range of Jaccard's similarity coefficient was found to vary from 0.46 (TGMS-7 and TGMS-16) to 0.97 (TGMS-11 and TGMS-12). The UPGMA based dendrogram constructed using Jaccard's similarity coefficient of SSR marker data divided eighteen lines into three clusters. Cluster strength varied from minimum of two members to maximum of eleven members in different clusters. Clustering patterns in general revealed that lines with same morphological traits and spikelet fertility and sterility did not occupy the same cluster. The marker based clustering pattern therefore did not show strong correlation with morphological data.


(M. K. Nautiyal)
Advisor


(Pardeep Kumar)
Author

नाम	: प्रदीप कुमार	परिचयांक संख्या	: 44160
प्रवेश वर्ष एवं षट्मास	: 2012-13, प्रथम	उपाधि	: पीएचडी
प्रमुख विषय	: आनुवंशिकी एवं पादप प्रजनन	विभाग	: आनुवंशिकी एवं पादप प्रजनन
गौण विषय	: आण्विक जीव विज्ञान और जैव प्रौद्योगिकी		
सलाहकार	: डॉ० एम०के०नौटियाल		

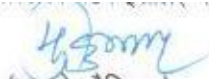
शोध शीर्षक : “प्रकाश संवेदी आनुवंशिक नरबन्ध वंशक्रमों का रूपात्मक व आण्विक मूल्यांकन एवम् चावल में उनके संकरओज संयोजन (ओराइजा सटाइपा एल.)”

सारांश

वर्तमान जांच के अनुसार एस०एस०आर० प्राइमरों को अलग-अलग रूपात्मक लक्षण और आण्विक विविधता के विलेषण के लिए अट्टारह TGMS वंशक्रमों का मूल्यांकन करने के लिए और इन पंक्तियों के लिए बन्ध तापमान को निर्धारित करने के लिए लिया गया था। इन अट्टारह TGMS वंशक्रमों का चार परीक्षकों के साथ क्रॉस किया और विभिन्न उपज के लक्षणों के योगदान के लिये और उपज के लिए सबसे उत्तम क्रॉस की पहचान की गई। खरीफ 2013-14 के दौरान चावल के चार चेकों के साथ बहत्तर एफ 1 एवं अट्टारह TGMS वंशक्रमों का गो०ब०पंत कृषि एवं प्रौद्योगिक विविद्यालय, पंतनगर में नार्मन ई बोरलॉग फसल अनुसंधान केन्द्र में तीन अनुकरण के साथ पूर्णतया यादृच्छिक ब्लॉक अभिकल्पना में निर्धारित किया गया था।

भिन्नता का विलेषण क्रॉस और वंशक्रम, एक्स परीक्षक प्रभाव में सभी वर्णों के लिए महत्वपूर्ण पाया गया था वंशक्रमों में सभी लक्षण, परीक्षक के लिए महत्वपूर्ण अंतर पौधे की ऊँचाई, प्रति स्टाइकलेट उर्वरता और 1000 दानों के वजन को छोड़कर महत्वपूर्ण अन्तर जैसे पुष्पगुच्छ लम्बाई, पुष्पगुच्छ प्रति पौधा संख्या, पुष्पगुच्छ प्रति दाना संख्या में दिखाया। चार वंशक्रमों TGMS-14, TGMS-17, TGMS-10 और TGMS-18 विभिन्न लक्षणों के लिए सबसे अच्छे सामान्य संयोजक थे। वंशक्रमों के बीच सामान्य संयोजन क्षमता पट और परीक्षकों जैसे पंत धान-4, पंत धान-2 और UPRI-93-287 आर विभिन्न गुणों के लिए अच्छे सामान्य संयोजक थे। चिन्हक संगठन अध्ययन के आधार पर इन पंक्तियों के बीच केवल दो वंशक्रम इस नंबर के लिए अच्छे TGMS वंशक्रमों की पहचान के लिए उपयोगी हो सकता है। केवल आर एम 276 चिन्हक द्वारा TGMS-10 और TGMS-17, 150 bp विष्ट पट्टी के साथ थे जो एक चिन्हक द्वारा विष्ट पट्टी से पता चल लक्षण और अधिक उपज प्राप्त करने के लिए संकर विकास में इस्तेमाल कर सकते हैं। विष्ट संयोजन क्षमता प्रभाव और प्रति प्रदर्शन के आधार पर साधारण क्रॉस के लिए अलग गुणों का चयन किया गया था। उपज प्रति पौधा के लिए सबसे अच्छे तीन क्रॉस TGMS-14 x पंत धान-4, TGMS-9 x पंत बासमती-1 और TGMS-16 x UPRI-93-287 को संकरों के विकास के लिए इस्तेमाल किये जा सकते थे। मानक संकरओज के पाँच आर्थिक क्रॉस अर्थात् TGMS-14 x पंतधान-4, TGMS-16 x UPRI-93-287 आर, TGMS-17x UPRI-93-287 आर, TGMS-9 x पंत DDR बासमती-1 और 14 x UPRI-93-287 आर दानों को उपज के लिए उच्च विष्ट संयोजन क्षमता की ओर से प्रभाव प्रति प्रदर्शन दिखाया है। सामान्य संयोजन क्षमता के विवरण का अनुमान पुष्पगुच्छ लंबाई, पौधा ऊँचाई एवं पुष्पगुच्छ प्रति संख्या स्पाइकलेटस को छोड़कर अन्य योज्य के जीन की कार्यवाही को प्रधानता दिखाकर सभी गुणों के लिए विष्ट संयोजन क्षमता के विवरण की तुलना में कम थे TGMS वंशक्रमों की रूपात्मक और आण्विक परिणाम एक लक्षण के लिए नहीं बल्कि सभी लक्षणों के अलग-अलग गुणों के लिए और अलग-अलग वंशक्रमों के लिए अच्छा माना गया। अट्टारह TGMS वंशक्रमों अर्थात् TGMS-2, TGMS-3, TGMS-4, TGMS-5, TGMS-7, TGMS-8, TGMS-10, TGMS-11, TGMS-14 और TGMS-15 पंक्तियाँ, 28.53⁰ से 28.92⁰ से औसत तापमान पर बन्ध हो जाती है। इसलिए इन पंक्तियों को मई के अंतिम सप्ताह में पंतनगर की स्थिति में पूर्ण बन्ध प्राप्त करने के लिए किया जाएगा। इन्हें संकर बीज उत्पादन में इस्तेमाल किया जा सकता है।

2013-14 के दौरान अट्टारह वंशक्रमों के बीच में चार वंशक्रम TGMS-1, TGMS-6, TGMS-9 और TGMS-18 दोनों मुख्य मौसमों में उपजाऊ स्पाइकलेटस दिखाया लेकिन 2014 के प्रतिकूल मौसम में पूर्ण बन्ध बालो दिखायी दी। आण्विक आनुवंशिक विविधता समंक के आधार पर सेंटालिस विकल्पी की कुल एक जोड़ी प्राइमर के लिए छोड़कर बहुरूपी होना पाया गया है, जो सभी चावल के अट्टारह TGMS वंशक्रमों की वर्तमान विलेषण में सोलह जोड़े एस०एस०आर० प्राइमरों का उपयोग प्रवर्धित था। प्राइमर प्रति विकल्पी की औसत संख्या अलग-अलग वंशक्रमों में 2.93 पाया और तेरह दुर्लभ आनुवंशिक विकल्पी थे, जबकि विकल्पी की सीमा 2-5 थी। इन सोलह जोड़े एस०एस०आर० प्राइमर के लिए बहुरूपी जानकारी (पी०आई०सी०) 0.17-0.37 भिन्न थी अर्थात् आसत 0.25 थी और जेकार्ड की समानता गुणांक की सीमा के साथ 0.46-0.97 बताया गया। UPGMA पर आधारित वृक्षम एस०एस०आर० चिन्हक समंक की जेकार्ड की समानता गुणांक तीन समूहों के अट्टारह वंशक्रमों में विभाजित कर प्रयोग किया। अलग-अलग समूहों में ग्यारह सदस्यों की अधिकता के लिए सामान्य रूप से क्लस्टरिंग पैटर्न स्पाइकलेटस प्रजनन और बन्धता के साथ वंशक्रमों के एक ही समूह पर कोई प्रदर्शन नहीं था। चिन्हक आधारित क्लस्टरिंग पैटर्न इसलिए मात्रात्मक समंक के साथ सकारात्मक सम्बन्ध नहीं दिखाता है।


(एम०के०नौटियाल)
सलाहकार


(प्रदीप कुमार)
लेखक